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(57) Abstract

The invention relates to methods of detection of bacteria of the genus Shigella and/or Enteroinvasive *E. coli* (EIEC) by use of a set of nucleic acid probes. The invention further relates to a set of Shigella specific chromosomal sequences and fragments and to probes derived from the Shigella specific fragments. Additionally, probes were derived from a sequence from the Shigella ompA gene. In particular, a series of probes, each approximately 40 nucleotides in length, were designed having specificity for Shigella or for Shigella and Enteroinvasive *E. coli*, and having utility in nonisotopic test formats which require amplification to achieve high sensitivity. Specific hybridization probe sets which are capable of detecting substantially all clinically significant serotypes of Shigella, as well as enteroinvasive strains of *E. coli*, are disclosed.

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NUCLEIC ACID PROBES FOR THE DETECTION OF SHIGELLA

Description

Background of the Invention

The genus Shigella includes four species (major serogroups): S. dysenteriae (Grp. A), S. flexneri (Grp. B), S. boydii (Grp. C) and S. sonnei (Grp. D) as classified in Bergey's Manual for Systematic Bacteriology (N.R. Krieg, ed., pp. 423-427 (1984)). These serogroups are further subdivided into serotypes (Table 1). The genera Shigella and Escherichia are phylogenetically closely related. Brenner and others have suggested that the two are more correctly considered sibling species based on DNA/DNA reassociation studies (D.J. Brenner, et al., International J. Systematic Bacteriology, 23:1-7 (1973)). These studies showed that Shigella species are on average 80-89% related to E. coli at the DNA level. Also, the degree of relatedness between Shigella species is on average 80-89%. Shigella boydii serotype 13 is atypical in that it is only 65% related to other Shigella serotypes and Escherichia.

The genus Shigella is pathogenic in humans; it causes dysentery at levels of infection of 10 to 100 organisms. By contrast, the majority of E. coli

(9000 O:H serotypes) are not associated with diarrheal disease. Pathogenic E. coli serotypes are collectively referred to as Enterovirulent E. coli (EVEC) (J.R. Lupski, et al., J. Infectious Diseases, 157:1120-1123 (1988); M.M. Levine, J. Infectious Diseases, 155:377-389 (1987); M.A. Karmali, Clinical Microbiology Reviews, 2:15-38 (1989)). This group includes at least 5 subclasses of E. coli, each having a characteristic pathogenesis pathway resulting in diarrheal disease. The subclasses include Enterotoxigenic E. coli (ETEC), Verotoxin-Producing E. coli (VTEC), Enteropathogenic E. coli (EPEC), Enteroadherent E. coli (EAEC) and Enteroinvasive E. coli (EIEC). The VTEC include Enterohemorrhagic E. coli (EHEC) since these produce verotoxins.

The pathogenesis of Enteroinvasive E. coli is very similar to that of Shigella. In both, dysentery results from invasion of the colonic epithelial cells followed by intracellular multiplication which leads to bloody, mucous discharge with scanty diarrhea.

Thus, detection of Shigella and EIEC is important in various medical contexts. For example, the presence of either Shigella or EIEC in stool samples is indicative of gastroenteritis, and the ability to screen for their presence is useful in treating and controlling that disease. Detection of Shigella or EIEC in any possible transmission vehicle such as food is also important to avoid spread of gastroenteritis.

Currently, presence of Shigella in stool samples is detected by cultivating an appropriately prepared sample on microbiological media under conditions favorable for growth of those bacteria. The resulting colonies are then examined for microbiological and biochemical characteristics, a process that typically takes at least three days and does not permit processing large numbers of samples. However, hospitals do not test for the presence of EIEC in stool because of the difficulty of serotyping which is necessary to identify the EIEC among the numerous, non-pathogenic E. coli normally present in stool.

Summary of the Invention

The present invention relates to methods of detection of bacteria of the genus Shigella and/or Enteroinvasive E. coli (EIEC) by use of a set of nucleic acid probes. The invention further relates to a set of Shigella specific chromosomal sequences and fragments, which were isolated by subtractive hybridization against non-Shigella DNA, and to probes derived from the Shigella specific fragments. Additionally, probes were derived from a sequence from the Shigella ompA gene. In particular, a series of probes, each approximately 40 nucleotides in length, were designed having specificity for Shigella or for Shigella and Enteroinvasive E. coli, and having utility in non-isotopic test formats which require amplification to achieve high sensitivity. In addition, specific hybridization

probe sets were developed which are capable of detecting substantially all clinically significant serotypes of Shigella, including S. sonnei, S. flexneri, S. boydii, and S. dysenteriae, as well as enteroinvasive strains of E. coli.

Probes or probe sets of the present invention can be used in a number of hybridization formats for the detection of Shigella species and/or Enteroinvasive E. coli. For example, the presence of one or more Shigella species and/or one or more species of EIEC in a sample can be determined by lysing the cells in the sample, contacting the sample with a DNA probe set under conditions suitable for hybridization of the probes with Shigella and/or EIEC DNA, capturing the hybrids formed between the probes and the sample DNA, and detecting the hybrid complexes by a suitable method as an indication of the presence of Shigella or EIEC in the sample.

Brief Description of the Figures

Figure 1 is a flow chart illustrating a strategy for the isolation of Shigella specific DNA sequences from "target" DNA and of Shigella specific fragments from a library of target DNA clones.

Figure 2 illustrates the nucleotide sequence (SEQ ID NO:1) of Shigella specific fragment NT6 and some flanking sequence, and the locations and sequences of probes 1500 (SEQ ID NO:14), 1501 (SEQ ID NO:15) and 1911 (SEQ ID NO:16), which are derived from these sequences.

Figure 3 illustrates the nucleotide sequence of Shigella specific fragment NT11-2 (SEQ ID NO:2), and the locations and sequences of probes 1682 (SEQ ID NO:17), 1683 (SEQ ID NO:18), 1708 (SEQ ID NO: 19), and 1709 (SEQ ID NO:20), derived from the fragment.

Figure 4 illustrates the nucleotide sequence of Shigella specific fragments NT14 (SEQ ID NO:4) and NT15 (SEQ ID NO:3), comprising a version of the Class III repeat isolated from S. sonnei, and the location and sequence of probes 1864 (SEQ ID NO:22) and 437 (SEQ ID NO:21), derived from these fragments. The sequences of three versions of the Class III repeat isolated from E. coli (E.c. 1; SEQ ID NO:5; and E.c. 2; SEQ ID NO:6 and SEQ ID NO:7) and S. flexneri (S.f.; SEQ ID NO:8 and SEQ ID NO: 9) are also shown.

Figure 5 illustrates the sequence of Shigella specific fragment NT18-1a (SEQ ID NO:10), and the location and sequence of probes 1712 (SEQ ID NO: 23) and 1713 (SEQ ID NO: 24), derived from the fragment.

Figure 6 illustrates the sequence of Shigella specific fragment NT19-2 (SEQ ID NO: 11), and the location and sequence of probes 1684 (SEQ ID NO: 25) and 1685 (SEQ ID NO: 26), derived from the fragment.

Figure 7 illustrates a portion of the sequence of the S. dysenteriae (S.d.) ompA gene (SEQ ID NO: 12), and the location and sequence of probes 1706 (SEQ ID NO: 27) and 1707 (SEQ ID NO: 28), derived from the ompA gene sequence. The E. coli (E.c.)

ompA gene sequence (SEQ ID NO: 13) corresponding to the same region is shown for comparison.

Detailed Description of the Invention

Previous investigators interested in developing specific probes for Shigella have targeted the virulence plasmid. Both Shigella and EIEC harbor a single copy virulence plasmid approximately 140 MD (215 kilobasepairs) in size which is necessary for invasion (T.L. Hale, Infection and Immunity, 40:340-350 (1983)). For example, U.S. Patent No. 4,816,389 (Sansonetti et al.) discloses a 27 kilobasepair (kb) region of the virulence plasmid proven necessary for invasion. These investigators have shown that the virulence plasmid, which exists as one copy per bacterium, is unstable; regions of the plasmid may be deleted. Shigella and EIEC strains, which have been stored or passaged in the laboratory, frequently are found to contain virulence plasmids of reduced size (C. Sasakawa, et al., Infection and Immunity, 51:470-475 (1986); A.T. Maurelli, et al., Infection and Immunity, 43:397-401)). The 27 kb region from which the probes of Sansonetti et al. have been derived has been shown to be one of the unstable segments (P.K. Wood, J. Clinical Microbiology, 24:498-500 (1986)). Strains of Shigella or EIEC which do not contain a portion of the 27 kb target region are not detected by the probe and are incorrectly identified as non-Shigella or non-EIEC.

Moreover, the 27 kb probe region contains insertion element 1 (IS1) which is ubiquitous among the Enterobacteriaceae (M. Venkatesan, et al., J. Clinical Microbiology, 26:261-266 (1988)). It also contains at least one copy of insertion element 600 (IS600; S. Matsutani, et al., J. Molecular Biology, 196:445-455 (1987)), which occurs frequently in both pathogenic and non-pathogenic representatives of E. coli, as well as in Shigella and EIEC (unpublished result). The presence of these broadly distributed insertion elements and the large size of the probes designed from the 27 kb region decrease the utility of these probes in non-isotopic test formats which require amplification in order to achieve high sensitivity.

In contrast, the present invention relates to probes and probe sets which are (1) developed from Shigella specific fragments derived from chromosomal sequences of Shigella and (2) moderate in size, each probe being approximately 40 bases in length. The increased stability of the chromosomal sequences detected by the probes compared to sequences of the virulence plasmid can result in increased reliability of detection. Furthermore, moderately sized probes have utility in non-isotopic test formats which require amplification to achieve high sensitivity. Both the Shigella specific fragments and the probes derived from them are also useful as hybridization probes in other formats. Some of the fragments derived from the Shigella chromosome are

also present in an episomal location on the invasion plasmid or another plasmid.

In one embodiment, the invention features a nucleic acid probe set consisting essentially of nucleic acids with sequences that are:

a. derived from the chromosomal sequence of representative bacteria of the species Shigella sonnei (ATCC 29930, designated type strain) and Shigella flexneri type 2a (ATCC 29903, designated type strain) but are less than the entire chromosomal sequence of these bacteria;

b. capable of hybridizing to DNA of members of the four known Shigella species and to Enteroinvasive E. coli (EIEC);

c. not capable of hybridizing or only weakly hybridizing to DNA of bacteria that are in neither the genus Shigella nor the group EIEC.

As used herein, a sequence fragment or oligonucleotide that is "derived from a chromosomal sequence" is a natural, engineered or synthetic molecule having a sequence which is identical or complementary to a chromosomal sequence or is identical or complementary to a variant of the chromosomal sequence. A sequence fragment or oligonucleotide which is identical or complementary to a variant of a selected chromosomal sequence (i.e., the variant differs in sequence from the

chromosomal sequence) is a homologue of the sequence or oligonucleotide which is identical or complementary, respectively, to the selected chromosomal sequence. This type of homologue will have a nucleotide sequence substantially similar to a chromosomal sequence and will retain the desired function (will be able to hybridize to substantially the same nucleic acids as the sequence fragment or oligonucleotide which is identical or complementary to that chromosomal sequence under similar hybridization conditions) of the fragment or oligonucleotide which is identical to the selected chromosomal sequence. A homologue may differ from the chromosomal sequence in sequence and/or may contain modified nucleotides or nucleotide analogs (e.g., phosphorothioates, methylphosphonates). A homologue must be able to hybridize to the same nucleic acid as the sequence fragment or oligonucleotide which is identical or complementary to that chromosomal sequence under similar hybridization conditions. It is well known to those skilled in the art that either strand of a double-stranded DNA sequence can serve as the target for a complementary probe. The complement of a given probe is expected to have a substantially similar hybridization pattern, under similar hybridization conditions. The probes may be DNA or RNA or modified DNA or RNA.

Nucleic acid fragments or oligonucleotides containing sequences derived from a chromosomal sequence, their homologues, and complements of all

of the foregoing may be used as probes. For example, the Shigella specific fragments, portions thereof and oligonucleotides from the Shigella specific fragments may be used as hybridization probes.

In one embodiment, either of two probe sets of synthetically produced nucleic acid probes (each approximately 40 nucleotides long) will detect substantially all clinically significant serotypes of Shigella, including S. sonnei, S. flexneri, S. boydii and S. dysenteriae. Clinically significant serotypes or isolates are those which are human pathogens. In addition, these probe sets recognize some or all enteroinvasive strains of E. coli, exclusive of other enteric bacteria tested, with the exception of Escherichia fergusonii.

The invention further relates to methods of detecting Shigella species or EIEC in a sample. For example, one or more Shigella serotypes and/or EIEC present in a sample can be detected by lysing the cells in the sample; contacting the sample with a nucleic acid probe or probes of the present invention under conditions that allow the probes to hybridize to Shigella and EIEC DNA in the sample, thus forming hybrid nucleic acid complexes; isolating hybrid nucleic acid complexes formed between the probes and DNA in the sample, and detecting the hybrid nucleic acid complexes as an indication of Shigella or EIEC in the sample. For example, clinical (e.g., stool), environmental (e.g. water), or food specimens may be subjected to such a

procedure to ascertain the presence of Shigella and/or EIEC species in the sample.

In preferred embodiments of the method, one or more pairs of probes are selected as a capture and detector probe pair for use in a dual probe liquid hybridization format. These probes can be produced synthetically by chemical or enzymatic synthesis methods. They may be produced as part of a larger molecule. For example, the capture probe can be tailed with 150-200 deoxyadenosine triphosphate (dATP) residues using the enzyme terminal deoxynucleotidyl transferase. The detector probe can be incorporated into an amplification/detection system, such as a biotin-streptavidin-alkaline phosphatase system. Both the capture and detector probes are then allowed to hybridize to the target nucleic acid in a background of competitor nucleic acid from the sample. The hybrid products can be captured out of the mixture by magnetic beads complexed with tails of deoxythymidine monophosphate generally 14 residues longer. The captured hybrids (i.e., those affixed to or captured on the magnetic beads) are detected by means of the amplification/detection system selected.

Isolation of Shigella-Specific Fragments

Shigella-specific genomic sequences were isolated by subtractive hybridization using biotin-streptavidin agarose affinity column chromatography methods described by Langer et al. (Langer, P.R. et al., Proc. Natl. Acad. Sci. USA,

78:6633-6637 (1981) and Welcher et al. (Welcher, A.A. et al., Nucleic Acids Res., 14: 10027-10044 (1986)), each of which is incorporated herein by reference. The method is outlined in Figure 1.

For example, a mixture of competing DNAs, such as the complex DNA competitor mix described in Figure 1 can be used. In general, a complex DNA competitor mix will contain an excess of DNA (relative to the target DNA) from one or more Enterohemorrhagic E. coli isolates, one or more Enterotoxigenic E. coli isolates, and one or more non-pathogenic E. coli isolates. The mix may further contain specific sequences already recovered or sequences designed to eliminate non-specific sequences. For example a plasmid containing a 17 kb region of the Shigella virulence plasmid such as pHS4033, Class III repeat DNA, or M13mp18 RF DNA may be included.

The particular complex DNA competitor mix used contained a 6-fold excess by weight of DNA (relative to the target DNA) from each of Enterohemorrhagic E. coli isolate IG 3040, Enterotoxigenic E. coli isolate IG 3060, and non-pathogenic E. coli (YMC). The mix further contained DNA, in amounts equal to the target DNA by weight, from each of the following: a pBR322 clone containing a 17 kb region of the Shigella virulence plasmid (plasmid pHS4033, Boileau, C.R. et al., J. Clin. Microbiol. 20(5): 959-961 (1984)), M13mp18 RF DNA, and the sequence of the Class III repeat (Class IIIR-IG900) cloned into pBR322. (The 1.3 kb Class III repeat and adjacent

chromosomal DNA was cloned into pBR322. The length of the insert was 3.5 kb.) The complete mixture was labelled with biotin-11-dUTP by the nick-translation method. The "target DNA" from which specific sequences were identified was isolated from a single Shigella species, digested with restriction endonuclease Sau3A, and end-labelled with ^{32}P in an end-filling reaction with DNA polymerase I.

The two DNA pools were combined such that the competing DNAs were at a 20-40 fold molar excess relative to the Shigella DNA. The mixture was denatured and hybridized in liquid at low stringency overnight. The hybridization buffer contained 0.75 M NaCl, 50 mM $NaPO_4$, 1 mM EDTA, 0.05% SDS and 40% formamide. The hybridization mixture then was passed over the streptavidin agarose column. Shigella DNA sequences that were sufficiently complementary to the competing DNAs to form hybrids under the conditions used were retained on the streptavidin agarose affinity column by virtue of the biotin incorporated into the competitor DNA. In contrast, sequences that were unable to form hybrids under those conditions were enriched in the nucleic acid fraction passing through the column. The latter sequences contain Shigella-specific sequences.

A small aliquot of the DNA enriched for Shigella-specific sequences (previously ^{32}P-labelled) was used to probe Shigella and E. coli DNAs of interest (0.1 μg of each DNA was spotted in a 3 μl volume on nitrocellulose). Hybridization

conditions were those described below in Example II for nick-translated fragments. Cross-hybridization to the competitor E. coli provided an indication that further enrichment of the Shigella "target" DNA was required. Typically, four cycles of competition hybridization versus the biotinylated E. coli competitor DNA were necessary to eliminate the cross-hybridization. This was accomplished by repeating the competition hybridization/affinity capture cycle, using the Shigella DNA which passed through the column during the previous cycle, as the starting material for the next cycle. In this way, the labeled Shigella target DNA was progressively enriched for Shigella-specific sequences in each cycle.

The nucleic acid which was enriched for Shigella-specific sequences was then used to probe a Sau3A library of the same Shigella isolate used as the "target DNA" in the subtractive hybridization; the library was constructed in the plasmid vector, pUC18. Inserts (fragments) from positive clones were purified from the vector, labelled with ^{32}P by nick-translation and used to probe mini-cyto-dot panels of inclusivity and exclusivity organisms as described in Examples I and II. A probe shows inclusivity toward an organism if DNA from the organism hybridizes to the probe, and shows exclusivity toward an organism if the probe does not hybridize (or if hybridization is barely detectable) to DNA from that organism under the particular hybridization conditions used.

In most cases, hybridization to inclusivity organisms was much stronger than hybridization to competitors or exclusivity organisms. When necessary, subcloning was done to remove the sequences which cross-hybridized to competitors. At this point, the fragments were labelled with ^{32}P by nick-translation and used to probe full inclusivity and exclusivity cyto-dot panels.

The inclusivity panels consisted of bacteria representing all known Shigella serotypes as well as Enteroinvasive E. coli which exhibit the same pathogenesis as Shigella. These organisms are listed in Tables 2 through 6. The exclusivity panels consisted of non-pathogenic E. coli, Enterotoxigenic E. coli, Enteropathogenic E. coli, Enterohemorrhagic E. coli, other Escherichia species and gram negative Enterobacteriaceae. The exclusivity organisms are listed in Tables 6 and 7a/7b.

In the Tables, under the conditions used, (-) indicates no signal, (+/-) or (-/+) was barely detectable, (+) indicates a weak but reproducible and readily detectable signal, (++) indicates a moderate signal, (+++) indicates a strong signal, and (++++) indicates a signal comparable to the positive control (DNA from the organism from which the fragment was isolated, or a known sequence identical to the probe being tested). The genomic (chromosomal) DNA fragments identified by the subtractive hybridization and refinement protocols

described above are referred to as Shigella specific fragments.

1. Shigella specific fragments NT19-2 (SEQ ID NO: 11) and NT18-1a (SEQ ID NO: 10) were isolated from a library of Shigella flexneri (ATCC 29903) genomic clones by probing the library with Shigella specific sequences isolated from S. flexneri (ATCC 22903) ^{32}P-labeled "target" DNA, using the "complex DNA competitor mix" described above in the subtractive hybridization steps.

2. Shigella specific fragments NT 6 (see SEQ ID NO:1), NT11-2 (SEQ ID NO:2), NT14 (SEQ ID NO: 4) and NT15 (SEQ ID NO:3) were isolated from a library of Shigella sonnei (ATCC 29930) genomic clones by probing the library with Shigella specific sequences isolated from S. sonnei (ATCC 29930) ^{32}P-labeled "target" DNA and using a single non-pathogenic E. coli (YMC) and 1 μg of pBR322 vector DNA as source of competitor DNA in the subtractive hybridization. In this case, avidin agarose rather than streptavidin agarose was used in the affinity column.

The hybridization results against inclusivity and exclusivity organisms for these Shigella-specific fragments are recorded in Tables 2-7. In addition, a set of ompA probes were developed from published

data (G. Braun, et al., Nucleic Acids Research, 10:2367-2378 (1982)) by comparison of the outer membrane protein gene sequence (ompA) of Shigella dysenteriae (SEQ ID NO:12) with that of E. coli (SEQ ID NO:13). The regions of ompA gene sequence which appeared most different between the two sequences were selected for the development of test probes. These were synthesized and assayed by hybridization analysis as described above.

Isolation of Shigella-Specific Oligonucleotides

The Shigella-specific fragments described above displayed the most marked specificity for inclusivity organisms of the Shigella DNA fragments analyzed. These fragments were sequenced and oligonucleotide probes useful as capture and detector probes were designed from these sequences. Oligonucleotide capture and detector probes were also designed from a fragment of the ompA sequence by comparative sequence analysis. Following synthesis, the oligonucleotides were end-labelled with ^{32}P and tested by cyto-dot hybridization analysis as described in Example I to ensure that they exhibited the desired inclusivity and exclusivity behavior or pattern. In several cases, an additional exclusivity panel was tested at this point (Table 8). This panel consisted of 4 μg DNA dots of gram positive and gram negative bacteria--including aerobic and anaerobic representatives commonly found in stool. The DNA was isolated by a protocol which makes use of glass

beads to physically disrupt the cell wall of bacteria. Each DNA was spotted on nitro-cellulose filters in a 3 μl volume; the DNAs were denatured, neutralized and fixed as described in Example I for the preparation of cyto-dot panels.

Description of Probes and Hybridization Behavior With Respect to Inclusivity and Exclusivity

Oligonucleotide probes were derived from the Shigella-specific fragments identified by affinity chromatography and from the ompA sequence. The sequence of each oligonucleotide probe and the Shigella-specific DNA fragment that each was derived from are listed in Table 9. The inclusivity and exclusivity hybridization behavior of the clones and subclones, and the oligonucleotides designed from these sequences are described below.

Fragment NT6 and Probes 1500, 1501, and 1911

NT6 (see SEQ ID NO:1) is a 124 bp Sau3A Shigella specific fragment. The sequence is repeated 6 times in Shigella sonnei (ATCC 29930) chromosomal DNA. It is also found in one or two copies on the virulence plasmid of other Shigella isolates. The entire fragment was sequenced (Figure 2). The first 124 bp of Figure 2 are from NT6 (see SEQ ID NO:1). The Sau3A site at the 3'-end of the NT-6 sequence is indicated. In this and other Figures, IUPAC conventions for referring to nucleotides and sequence ambiguities are used:

Unambiguous bases:	A, C, G, and T (or U)	
Two possible bases:	M	(A or C)
	R	(A or G)
	W	(A or T)
	S	(C or G)
	Y	(C or T)
	K	(G or T)
Three possible bases:	B	(C, G or T)
	D	(A, G or T)
	H	(A, C or T)
	V	(A, C or G)
Four possible bases:	N	(A, C, G or T)

Where there is a weak band on the sequencing gel, but no other band, the base is indicated by a lower case letter. Regions of ambiguous nucleotide order due to band compression are enclosed in parentheses.

Two oligonucleotides derived from NT6, each 35 bases long (1500, SEQ ID NO:14) and 1501, SEQ ID NO: 15), were designed and can be used as capture and detection probes (Table 9). A third probe, 40 bases long (1911, SEQ ID NO:16), was designed from NT6 and from additional sequence adjacent to the Sau3A NT-6 fragment (38 bp; see SEQ ID NO:1). This additional sequence was obtained from a clone isolated from an S. sonnei library using the NT-6 124 bp fragment as a probe. A sequencing primer internal to NT-6 was used for sequencing. Oligonucleotide 1911 was

designed and tested. The hybridization pattern of 1911 was identical to that of NT6, suggesting it is part of the repeated element.

The individual oligonucleotides were tested for hybridization to inclusivity and exclusivity organisms. In cases where all three probes displayed the same hybridization pattern, the results are not listed separately. The individual oligonucleotides, like the parent fragment, hybridized (using a + signal as the lower limit of hybridization) to all S. sonnei isolates tested, many S. dysenteriae and S. boydii, S. flexneri serotypes, including S. flexneri type 6 (Tables 2 through 5 and summary Table 10). The oligonucleotide probes derived from NT6 hybridized to all Enteroinvasive E. coli, but not to other classes of pathogenic E. coli, non-pathogenic E. coli or other organisms commonly found in stool (Tables 6-8).

Fragment NT11-2 and Probes 1682, 1683, 1708 and 1709

NT11-2 (SEQ ID NO:2) is a 796 bp Hha I subclone of an original Sau3A fragment (NT11) which was 3.5 kb in length. Fragment NT11-2 has been sequenced (Figure 3; SEQ ID NO:2). Two sets of oligonucleotide probes, useful as a capture/detector probe pair were designed from opposite ends of the fragment. Probes 1682 (SEQ ID NO:17) and 1683 (SEQ ID NO:18) are 41 nucleotides long (Table 9). Probes 1708 (SEQ ID NO:19) and 1709 (SEQ ID NO:20) are 35 and 36 nucleotides long, respectively. When used as

capture/detector probes, each capture probe can be synthesized with an additional three nucleotides (TAT) at the 3′ end to ensure efficient tailing by the enzyme terminal deoxynucleotidyl transferase. Tables 2 through 5 and summary Table 10 show that the two sets of oligonucleotides have different hybridization patterns. The cumulative hybridization pattern using the four probes together is the same as that of the parental fragment, NT 11-2, which hybridizes to all S. sonnei isolates, and to some S. dysenteriae, S. flexneri and S. boydii isolates. However, when used as capture/detector probe pairs (i.e., 1682 paired with 1683 or 1708 paired with 1709), certain serotypes would not be detected because one partner of each probe set does not hybridize to the isolate (e.g., S. dysenteriae types 9 and 10, S. boydii type 17). One of the four Enteroinvasive E. coli is detected strongly with 1708/1709 and weakly with 1682/1683. Two pathogenic E. coli isolates would be detected by 1708/1709 while only one of these isolates would be detected with 1682/1683. Apart from these pathogenic isolates, the oligonucleotides do not cross-hybridize to other non-pathogenic E. coli or other organisms commonly found in stool (Tables 6 and 7a).

Fragments NT14 and NT15, and Probes 437 and 1864

NT14 (SEQ ID NO:4)and NT15 (SEQ ID NO:3) are Sau3A fragments which are approximately 800 bp and 600 bp in size, respectively. The two fragments

have been sequenced and are portions of a highly repeated element which is 1.3 kb in length. One representative repeat each from S. sonnei (ATCC 29930) and S. flexneri (ATCC 29903) and two repeats from E. coli (IG900) were cloned and sequenced (Figure 4; S. sonnei repeat, SEQ ID NO: 3 and SEQ ID NO:4; E. coli repeat 1, SEQ ID NO:5; E. coli repeat 2, SEQ ID NO:6 and SEQ ID NO:7; S. flexneri repeat, SEQ ID NO:8 and SEQ ID NO:9). The repeat is highly conserved and has characteristics of a transposable element. Over 20 copies of the repeat sequence are present in the chromosome and virulence plasmid of Shigella. The repeat occurs in 1 to 3 copies in some E. coli competitors, but not in other bacterial species.

There are only a few differences between the E. coli and S. sonnei sequences shown in Figure 4. A 17 base oligonucleotide probe (probe 1864, SEQ ID NO: 22) was designed such that a single mismatch is located eight bases from either end of the probe (Table 9). This probe hybridizes strongly to the majority of Shigella and EIEC tested and does not cross-hybridize to competitor bacteria (Tables 2-8 and summary Table 10). A companion detector probe can be designed within the boundaries of the Class III repeat on either side of the specific capture probe, 1864. One such example is the complement of probe 437 (SEQ ID NO:21) (49 bases) for which inclusivity and exclusivity is listed in Tables 2 through 8. This probe hybridizes strongly to all serotypes of Shigella except S. boydii serotype 13.

Probe 437 hybridizes weakly to a number of non-pathogenic and pathogenic strains of E. coli, but does not hybridize to other Enterobacteriaceae tested. The hybridization signal with certain E. coli is due to the low copy number of the Class III repeat in this genus versus the high copy number of the repeat in the genus Shigella.

Two extra bases incorporated into the sequence of probe 437 as a result of sequencing errors in the S. sonnei sequence (a G and a T) have proved useful in decreasing the signal for E. coli isolates relative to Shigella strains. The 437 probe does not hybridize as well as expected to the positive control for S. sonnei or S. flexneri, suggesting that the two nucleotides in question are not present in the S. sonnei sequence. This observation also is likely to be related to copy number differences between the two genera.

Probe 1864 (SEQ ID NO:22) hybridized to all isolates of S. dysenteriae tested except for one isolate of serotype 1 (IG 826). However, serology on this isolate was not confirmed.

Fragment NT18-1a and Probes 1712 and 1713

NT 18-1a (SEQ ID NO:10) was subcloned from the original Sau3A fragment, NT18, in two steps. A PstI/SacI double digest of NT18 (1500 bp) yielded fragment NT18-1 (1250 bp), which was then restricted with HaeIII to generate NT18-1a (630 bp). Sequences related to fragment NT18 are also known to occur on a small multicopy plasmid which is distinct from the

215 kbp invasion plasmid. The sequence of NT18-1a is shown in Figure 5 (SEQ ID NO:10). Oligonucleotide probes 1712 (SEQ ID NO:23) and 1713 (SEQ ID NO:24), suitable as capture/detection probes, both 37 bases long, were designed from the sequence (Figure 5 and Table 9). The hybridization pattern (isolates detected) of the oligonucleotide probes was identical to the parental Shigella specific fragment NT18-1a with the exception of one S. flexneri isolate (Tables 2-5, 7b). This strain (IG 711) was detected by oligonucleotide probe 1713, but not by probe 1712. In a liquid hybridization assay when the two probes would be used as a capture/detection probe pair this organism would not be detected. Under the conditions used, the probes hybridize to 6/8 type 1 S. dysenteriae, to all S. flexneri isolates with the exceptions of three type 6 isolates, the IG711 isolate mentioned above, IG872, IG741, and IG709 (Tables 2 through 5 and summary Table 10). The probes do not detect Enteroinvasive E. coli, but cross-hybridize to one pathogenic E. coli under the conditions used. They do not cross-hybridize to non-pathogenic E. coli or other bacteria commonly found in stool (Tables 6 through 8).

Fragment NT19-2 and Probes 1684 and 1685

Fragment NT19-2 (388 bp; SEQ ID NO:11) is an RsaI subclone of the original Sau3A fragment which was 1070 bp in length. NT19-2 was sequenced (Figure 6; SEQ ID NO:11) and oligonucleotide probes 1684

(SEQ ID NO:25) and 1685 (SEQ ID NO:26), each 35 nucleotides long, were designed (Table 9). These probes are suitable as capture/detection probes. The hybridization patterns or spectrum of isolates detected by the individual oligonucleotides and the parental fragment are identical. Hybridization to some S. boydii, some S. sonnei and all S. flexneri except type 6 was observed (Tables 2-5, summary Table 10). The probes hybridize to one out of five Enteroinvasive E. coli, and do not cross-hybridize to other pathogenic E. coli, non-pathogenic E. coli or other bacteria commonly found in stool (Tables 6-8).

ompA Fragment and probes 1706 and 1707

Oligonucleotides 1706 (SEQ ID NO:27) and 1707 (SEQ ID NO:28) were designed from the published sequence of the outer membrane protein gene (ompA) of Shigella dysenteriae. Figure 7 shows the S. dysentaeriae ompA gene sequence from nucleotide position 893 through 1076, according to the numbering of Braun et al. (Nucl. Acids Res. 10(7): 2367-2378 (1982); SEQ ID NO:12). This region contains significant differences between the E. coli and S. dysenteriae ompA coding sequences. The sequence of the corresponding region of the E. coli ompA gene is shown for comparison (SEQ ID NO:13), and the positions of probes 1706 (SEQ ID NO:27) and 1707 (SEQ ID NO:28) are indicated in Figure 7.

Both oligonucleotides are 35 bases long (Table 9). Probe 1706 has 7 differences between the E.

coli and the S. dysenteriae sequence. The region from which probe 1707 was designed is 15 bases shorter in E. coli. Additionally, there are numerous differences in that probe site between E. coli and S. dysenteriae. These probes hybridize with S. dysenteriae types 1 and 2 and many S. boydii serotypes (Tables 2-5, summary Table 10). When probe 1707 is used as the specific capture probe and 1706 is used as the detector probe in liquid hybridization, no hybridization is anticipated to Enteroinvasive E. coli, other pathogenic E. coli, non-pathogenic E. coli and other bacteria commonly found in stool with the exception of Escherichia fergusonii (Tables 6-8).

Description of Probe Sets

Probe Set I

A desired inclusivity/exclusivity pattern may be achieved by use of various combinations of probes. One possible strategy involves pooling one or more combinations of probe pairs to make a probe set. For example, capture/detection oligonucleotides designed from fragments NT 6 (SEQ ID NO:1), NT 19-2 (SEQ ID NO:11) and the ompA gene (SEQ ID NO:12) may be used together as a probe set or combination for detection of substantially all clinically significant Shigella serotypes. One possible probe set comprises three capture/detection probe pairs, including probe pairs 1684/1685, 1707/1706, and a pair selected from probes

1500/1911/1501. This substantially inclusive probe set detects all Shigella serotypes except for S. dysenteriae type 10 and S. boydii type 13 (using a + signal as a lower limit for hybridization). In addition, this probe set does not cross-hybridize under the conditions used to any of the competitors tested except for Escherichia fergusonii.

The two Shigella serotypes that are not detected by probe set I under these conditions, are rarely isolated in the United States, as indicated by records of the Center of Disease Control. For example, out of a total of 167,915 cases of Shigella infection reported by the Center for Disease Control for the years 1976 through 1987, only two cases were identified as S. dysenteriae type 10 and three cases as S. boydii type 13.

In a capture/detection assay format, the more specific oligonucleotide of a capture/detector pair is preferred as the capture probe. In the case of the 1684/1685 probe set, either oligonucleotide may serve as the capture probe with equivalent hybridization results. However, in the case of the 1707/1706 probe set, oligonucleotide 1707 is preferred as the capture probe since it does not cross-hybridize to competitors. (Probe 1706 has (+/-) hybridization signal with certain competitors and a strong signal with an E. blattae isolate (Tables 6, 7b)).

In the case of the 1500/1911/1501 oligonucleotide probes, it is best to use either 1500 or 1911 as the capture probe. Probe 1501 has a

(+/-) hybridization signal with certain competitors (Table 8), but may be used as a detector probe with no adverse effects.

Probe Set II

An alternative probe set combines capture/detection oligonucleotide probe pairs from the Class III repeat (fragments 14 and 15; SEQ ID NO:4 and SEQ ID NO:3, respectively) and the ompA gene (SEQ ID NO:12). This substantially inclusive probe set 1707/1706 and 1864/437-complement) hybridizes to all Shigella except S. boydii type 13 and cross-hybridizes weakly to Escherichia fergusonii.

In the case of the capture/detector pair 1864/437-complement, it is best to use oligonucleotide 1864 as the capture probe since it is the more specific probe of the pair. Oligonucleotide probes designed from sequences to the left or right (in the 5′ or 3′ direction and from the same strand) of the sequences from which probe 1864 was derived may also serve as detector probes. The complement of probe 437 is one such example, and is expected to substantially retain the hybridization pattern of probe 437.

Use of probe combination II requires only four oligonucleotides instead of six, yet gives the desired inclusivity and exclusivity (substantially inclusive, in this case). In addition, the target of probe 1864 is present in multiple copies (20-30 copies), and therefore, allows for increased

sensitivity. However, carefully controlled hybridization conditions are necessary to maintain exclusivity with probe set II, since the specificity of probe 1864 depends on a single mismatch to differentiate between Shigella and E. coli which harbor the repetitive element (Class III repeat).

Table 12 lists the number of isolates expected to be detected by probe set I and probe set II in a capture/detection format using a (++) hybridization signal as the cut-off for detection. The results for probe sets I and II in a capture/detection format are expected to be the same when a (+) signal is used as the cut-off for detection.

Additional Probes

Other probes (double- or single-stranded nucleic acid fragments or oligonucleotides), probe sets or combinations may be derived from the Shigella specific fragments. These fragments or oligonucleotides (probes) "derived from the sequence of Shigella specific fragments", comprise nucleic acid sequences which are identical or complementary to a portion of the sequence of the Shigella specific fragments (and therefore to the Shigella chromosome). In some cases, only a portion of the probe may be identical to the sequence of the original Shigella specific fragment. Portions of a probe which are identical or complementary to the sequence of a Shigella specific fragment can be noncontiguous in the probe.

The preferred probes will retain features of the inclusivity, and if desired, the exclusivity of the Shigella specific fragments. A probe derived from a Shigella specific fragment which "substantially retains the inclusivity behavior of" a selected Shigella specific fragment hybridizes, under the same conditions, to at least one isolate of 90% or more of the (typed) serotypes to which the original fragment hybridizes. An original Shigella specific fragment which hybridizes to at least one isolate of one of the 35 serotypes listed in Table 1 is said to hybridize to that serotype. A probe which "moderately retains the inclusivity behavior of" a selected Shigella specific fragment hybridizes, under the same conditions, to at least one isolate of 83% or more, but less than 90%, of the serotypes to which the original fragment hybridizes. A probe which "partially retains the inclusivity behavior of" a selected Shigella specific fragment hybridizes, under the same conditions, to at least one isolate of 50% or more, but less than 83%, of the serotypes to which the original fragment hybridizes.

Exclusivity was determined using two sets of exclusivity organisms. The exclusivity organisms screened included 152 non-EIEC strains listed in Tables 6, 7A and 7B, and defined here as non-EIEC Enterobacteriaceae exclusivity organisms. In addition, the 91 strains listed in Table 8, comprise a second set of exclusivity organisms defined here as exclusivity organisms commonly found in stool.

A probe derived from a Shigella specific fragment which has "improved" exclusivity behavior for a given set of exclusivity organisms (e.g., non-EIEC Enterobacteriaceae) is one for which all of the exclusivity organisms of that set have been screened in the dot blot format, and which detects (hybridizes with a signal of (+) or better) fewer exclusivity organisms of that set than the Shigella specific fragment from which it is derived, under the same hybridization conditions. A probe derived from a Shigella specific fragment which, for a given set of exclusivity organisms, "substantially retains" the exclusivity behavior of the fragment from which it is derived, is one for which 90% or more of the exclusivity organisms of that set have been screened in the dot blot format, and which has substantially the same or identical exclusivity behavior under the same hybridization conditions. In particular, a probe which will detect no more than 13 strains of a set of exclusivity organisms which are not detected by the fragment from which it is derived, and which may or may not detect the exclusivity organisms which are detected by the original fragment, is defined as one which "substantially retains" the exclusivity behavior of that fragment. It will be appreciated that a probe for which exclusivity has been determined for 100% of a given set of exclusivity organisms, but which detects the same number of exclusivity strains or more (but not more than 13 additional strains) of

the exclusivity organisms as the original fragment falls in this latter category.

Furthermore, a probe for which exclusivity has not been determined for 100% of the organisms may be shown to have "improved" exclusivity behavior. For example, probes 1684 (SEQ ID NO:25) and 1685 (SEQ ID NO:26), for which the exclusivity for 4 non-EIEC Enterobacteriaceae has not been determined, do not detect two strains in Table 6 which are detected by NT19-2 (SEQ ID NO:11). If it is determined that these probes do not detect three or four of the strains not tested, then they will have improved exclusivity behavior, although they are presently classified as substantially retaining the exclusivity of NT19-2. Thus, the two classifications are not mutually exclusive.

Homologues of fragments and oligonucleotides derived from Shigella specific fragments, which hybridize to substantially the same serotypes as the fragments and oligonucleotides derived from Shigella specific fragments under the same hybridization conditions, can also be used. Homologues of a sequence fragment or oligonucleotide derived from a Shigella specific sequence will be identical or complementary to all or part of a variant of the sequence of a Shigella specific fragment.

For example, oligonucleotide probes, typically from about 10 nucleotides in length up to about 50 nucleotides in length, comprising sequences identical to a portion of a Shigella specific fragment can be designed. However, an

oligonucleotide probe may be longer than 50 nucleotides. Larger fragments comprising a sequence identical to a portion of a full length fragment can be prepared by restriction digestion of an isolated clone, exonuclease digestion, by the polymerase chain reaction using selected primers, or other suitable methods, for example.

The additional probes derived from the Shigella specific fragments, complements or homologues thereof, can be screened using a dot blot format (such as the cyto-dot or DNA dot blot formats of the Examples). These additional fragments or oligonucleotide probes can be used alone or in various probe pairs or probe combinations, or in addition to a selected probe, probe pair or combination from Table 9. The additional probes can also be used as alternatives to the probes listed in Table 9. For example, another probe derived from Shigella specific fragment NT11-2 (SEQ ID NO:2) could be used together with probe 1682 (SEQ ID NO:17) in place of probe 1683 (SEQ ID NO:18). For use in a capture/detection format, the probe would be derived from the same strand of the Shigella specific fragments as probe 1682. A probe which substantially retained the inclusivity pattern of NT11-2 could be selected, for example. Furthermore, probes of the present invention can be used in combination with other probes for Shigella, enteroinvasive E. coli, or other organisms (e.g., Salmonella, Campylobacter, etc.).

It will be appreciated that the signal for the recommended probe sets may be increased by using additional probes. Additional probes may be selected from the probes listed in Table 9, oligonucleotide probes derived from the large Shigella specific fragments disclosed, the homologues and complements of any of the foregoing, or other suitable probes. Although some probes are preferred as detection probes in a capture-detection probe format due to hybridization with exclusivity organisms, each probe may be used as either a capture or detection probe.

Inclusivity and Exclusivity Patterns

Different inclusivity and exclusivity patterns can be obtained using selected combinations of probes. Furthermore, inclusivity and exclusivity behavior may be modulated by hybridization conditions, and/or by taking a specific level of hybridization as the cut-off. For example, in Table 10, a (+) signal, which is a weak but reproducible and readily detectable signal, is used as the cut-off for inclusivity or detection in the dot blot format (cyto-dot or DNA dot format).

In Table 11, a (++) cut-off is used. In Table 11, the number of isolates of each serotype or untyped isolate to which the probes NT6, NT11-2, NT18-1a, NT19-2, hybridized with a signal of at least (++) is indicated. In addition, the expected number of isolates of each serotype or untyped isolates to which probe pairs selected from

1911/1500/1501, 1682/1683, 1708/1709, 1712/1713, 1684/1685, or 1706/1707 are expected to hybridize with a signal of at least (++) in a capture/detection format are also indicated. (ND indicates that hybridzation was not determined for a particular isolate.)

Unless indicated otherwise herein, a probe which "detects" or for "the detection" of an isolate or serotype is one which gives at least a (+) signal under the hybridization conditions used with the isolate or with an isolate of a specific serotype. An individual probe (fragments or oligonucleotides), probe pair or probe set (a combination of probes and/or probe pairs) which, under the conditions used in the dot blot format, detects (hybridizes with a signal of at least +) at least one isolate of 90% or more of the serotypes listed in Table 1 is defined as a "substantially inclusive" probe, probe pair, or probe set. Similarly, a probe, probe pair, or probe set which, under the conditions used in the dot blot format, detects at least one isolate of 83% or more, but less than 90%, of the serotypes listed in Table 1 is a moderately inclusive probe, probe pair or probe set. A probe, probe pair or probe set which, under the conditions used in the dot blot format, detects at least one isolate of 50% or more, but less than 83%, of the serotypes listed in Table 1 is a partially inclusive probe, probe pair or probe set.

For example, probe sets I and II described above are substantially inclusive probe sets. Based

on the dot blot data (see summary Table 10), these combinations are expected to detect at least one member of 33 and 34 out of 35 serotypes, respectively in a capture/detection format (94.2% and 97.1%). In fact, each of these probe sets is expected to detect every isolate tested of the serotypes detected.

Individual probes such as oligonucleotides 1900, 1500 or 1501, which detect at least one member of 60% of the serotypes listed in Table 1, or fragment NT-6, which detects approximately 68% of the serotypes listed in Table 1 (see Table 10), would be considered partially inclusive probes. Probe 1864 is a substantially inclusive probe. Partially, moderately and substantially inclusive probes may be combined with each other or with other probes into appropriate pairs or sets to achieve a desired inclusivity pattern.

As stated above, the exclusivity organisms screened include the 152 non-EIEC strains listed in Tables 6, 7A and 7B, and defined here as non-EIEC Enterobacteriaceae exclusivity organisms. In addition, the 91 strains listed in Table 8, define a second set of exclusivity organisms defined here as exclusivity organisms commonly found in stool. An individual probe (fragments or oligonucleotides), probe pair or probe set (a combination of probes and/or probe pairs) which, under the conditions used in the dot blot format, does not detect (hybridizes with a signal less than +) any of the 152 non-EIEC strains listed in Tables 6, 7A and 7B is defined as

an "exclusive" probe, probe pair or probe set with regard to the non-EIEC Enterobacteriaceae exclusivity organisms. A probe, probe pair, or probe set which, under the conditions used in the Tables, detects (hybridizes with a signal of (+) or better) 10% or less of the 152 non-EIEC strains listed in Tables 6 and 7A and B is defined as a "substantially exclusive" probe, probe pair or probe set with regard to these E. coli and Enterobacteriaciae exclusivity organisms, while a probe which detects 20% or less of the 152 non-EIEC strains listed in Tables 6 and 7A and B is defined as "moderately exclusive" of the non-EIEC Enterobacteriaceae exclusivity organisms. A probe which is "exclusive" also meets the criteria for a moderately or substantially exclusive probe.

A probe, probe pair or probe set which, under the conditions used in the dot blot format, does not detect (hybridizes with a signal less than +) any of the 91 strains commonly found in stool and listed in Table 8 is defined as an "exclusive" probe, probe pair or probe set with regard to the exclusivity organisms commonly found in stool. A probe, probe pair, or probe set which, under the conditions used in the Tables, detects (hybridizes with a signal of (+) or better) to 10% or less of the 91 strains listed in Table 8 is defined as "substantially exclusive" of the exclusivity organisms commonly found in stool. Thus, a probe that is exclusive of the organisms in Table 8 is also substantially exclusive of the same organisms.

For example, probes 1500, 1501 or 1911 are exclusive of the non-EIEC Enterobacteriaceae of Tables 6 and 7, as well as of the strains commonly found in stool. Probes 1684 and 1685 did not detect any of the non-EIEC tested; however, 4 organisms (2.6%) were not tested. Thus, 1684 and 1685 are known to be substantially exclusive. If none of these 4 organisms is detected, then these probes would be exclusive. Probe set I, when used in a capture/detection format, is expected to detect 2 of the strains from Table 7 (Escherichia fergusonii). However, four strains from Table 7 were not tested (ND). Therefore, probe set I detects at most 3.9% (6/152) of the non-EIEC Enterobacteriaceae, and is thus "substantially exclusive" of the non-EIEC Enterobacteriaceae exclusivity organisms. Probe set I is also exclusive of the exclusivity strains commonly found in stool (listed in Table 8).

Probe 437 detects about 9.9% (15) of the non-EIEC organisms; however, 10/152 of the non-EIEC Enterobacteriaceae were not screened. Thus, the exclusivity of this probe for the non-EIEC Enterobacteriaceae is between 16.4% (moderately exclusive) and 9.9% (substantially exclusive). However, in a capture/detection format, probe set II, which may use the complement of 437, is substantially exclusive of the non-EIEC Enterobacteriaceae exclusivity organisms and exclusive of the exlusivity strains commonly found in stool due to the behavior of probe 1864. The expected behaviour of probe pairs of probe sets I

and II in a capture detection format with respect to EIEC, non-EIEC Enterobacteriaceae, and strains commonly found in stool is summarized in Table 13, in which a (+) signal is used as the cut-off for detection. (In Table 13, as in Tables 11 and 12, any pair selected from probes 1500, 1501 and 1911 displays the behavior indicated.)

Assay Formats for Probes

Probes, probe pairs, and probe sets can be used in a variety of hybridization assay formats. Such hybridization assays include solution hybridization assays in which the sequences to be detected and the probes are free in solution, or assays in which one of the sequence or probe is fixed to a solid support. Shigella specific fragments, portions thereof, oligonucleotide probes derived from the fragments, complements, or homologues can be used in dot blot formats or other appropriate hybridization-based assay formats. For example, the large fragments or portions thereof can be prepared as probes by nick translation or other suitable methods for filter hybridization (see e.g., U.S. Patent 4,358,535, Falkow et al.).

The probes can be used in suitable capture detection assay formats (see e.g., D.V. Morrissey, et al., Analytical Biochemistry, 181:345-359 (1989); W.R. Hunsaker, et al., Analytical Biochemistry, 181:360-370 (1989); H. Lomeli, et al., Clinical Chemistry, 35:1826-1831 (1989); Pritchard, C.G. and J.E. Stefano, Ann. Biol. clin. 48:492-497 (1990)).

In a capture/detector format, the probe pairs are preferably selected from non-overlapping portions of the same strand (a selected strand) of a Shigella specific fragment or a variant of a Shigella specific fragment. The probes can be separated by a distance consistent with activity in the selected assay. Thus, in a standard capture/detection format, the probes should be close enough that sample preparation does not separate the complementary sequences to the extent that the desired sensitivity of detection is compromised.

RNA probes may also be prepared. For example, probe nucleotide sequences can be incorporated into a fal-st MDV cDNA construct, and transcribed from linearized plasmid using T7 RNA polymerase. A detection probe prepared in this way can be used with one or more capture probes and amplified in Qβ replicase system (Pritchard, C.G. and J.E. Stefano, Ann. Biol. Clin. 48: 492-497 (1990)).

The oligonucleotide probes described or others based on the sequence of the Shigella specific fragments can be used in the polymerase chain reaction. A second oligonucleotide can be prepared from the opposite strand.

The present invention will now be illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLES

Example 1 Cyto-dot Panels

All cyto-dot panels were prepared by spotting approximately 1 X 10^8 cells of each bacterial isolate in a 5 μl volume onto nitrocellulose. The bacteria were lysed and the DNA was denatured by placing the nitrocellulose filters on 3MM paper wetted with 0.5 M NaOH and 1.5 M NaCl for 10 minutes. Following this treatment, the nitrocellulose filters were neutralized by placing them on 3MM paper wetted with 1 M Tris pH 7.5 and 1.5 M NaCl for 10 minutes. The latter neutralization step was repeated, and the DNA was fixed to the filters by baking under vacuum for 1-1.5 hours at 80 ^{o}C.

Example 2 Hybridization Conditions

The hybridization conditions for all nick-translated fragments were as follows: Prehybridization--was in 10X Denhardt's, 5X SET, 0.1 M phosphate buffer pH 7, 0.1% sodium pyrophosphate, 0.1% SDS for 3 hours at 65 ^{o}C. (Note that 20X SET is 3M NaCl, 0.4M Tris-HCl pH 7.5 and 20mM EDTA). Hybridization--was in 2X Denhardt's, 5X SET, 0.1 M phosphate buffer pH 7, 0.1% sodium pyrophosphate, 0.1% SDS and 1 X 10^6 counts of nick-translated probe per ml of hybridization solution. Hybridizations occurred overnight at 65 ^{o}C. The autoradiographs were exposed for 15 hours.

The hybridization conditions for all kinased oligonucleotides (except probe 1864, a 17 base (b) oligonucleotide) were as follows:

Prehybridization--was in 5X Denhardt's, 6X SET, 0.1 M phosphate buffer pH 7, 0.1% sodium pyrophosphate, 0.1% SDS for 3 hours at 60 ^{o}C. Hybridization--was in 1X Denhardt's, 6X SET, 0.1 M phosphate buffer pH 7, 0.1% sodium pyrophosphate, 0.1% SDS and 1 X 10^{6} counts per minute of kinased oligonucleotide probe per ml of hybridization solution. Hybridizations occurred overnight at 60 ^{o}C. The autoradiographs were exposed for 15 hours or 7 days. The data recorded in Tables 2-8 are from 7 day exposures The results of the two exposures were similar. The hybridization conditions for probe 1864 (17 b) were identical to those above except that the prehybridization, hybridization and wash temperatures were 50 ^{o}C rather than 60 ^{o}C.

TABLE 1

SIMPLIFIED OUTLINE OF SHIGELLA CLASSIFICATION

SPECIES	SEROLOGICAL SUBGROUP	SEROLOGICAL TYPE(S)
S. dysenteriae	A	1 through 10
S. flexneri	B	1 through 6
S. boydii	C	1 through 18
S. sonnei	D	1

TABLE 2 : HYBRIDIZATION RESULTS FOR SHIGELLA DYSENTERIAE SEROTYPES

Genus species Strain ID, serotype		NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Shigella dysenteriae																	
RF970 ,	1	-	-	-	-	-	-	-	-	-	-	+/-	-	++++	++++	++++	+++
RF952 ,	1	-	-	-	-	-	-	-	-	-	-	+/-	-	++++	++++	++++	+++
IG703 ,	1	-	-	-	-	-	-	-	++++	++++	++++	+/-	-	++++	++++	++++	+++
IG704 ,	1	-	-	-	-	-	-	-	++++	++++	++++	+/-	-	++++	++++	++++	+++
IG705 ,	1	-	-	-	-	-	-	-	++++	++++	++++	+/-	-	++++	++++	++++	+++
IG710 ,	1	-	-	-	-	-	-	-	++++	++++	++++	+/-	-	++++	++++	++++	+++
IG826 ,	1	-	-	-	-	-	-	-	++++	++++	++++	+/-	-	++++	++++	++++	-/+
IG828 ,	1	-	-	-	-	-	-	-	++++	++++	++++	+/-	-	++++	++++	++++	+++
IG774 ,	2	-	-	+++	+++	++++	-	-	-	-	-	+/-	-	++++	++++	++++	+++
IG725 ,	2	++	+++	+++	+++	++++	-	-	-	-	-	+/-	-	++++	++++	++++	++++
IG861 ,	3	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG940 ,	3	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG941 ,	3	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG942 ,	4	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG824 ,	4	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG862 ,	4	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG863 ,	5	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++

TABLE 2 : CONT'D

Genus species Strain ID, serotype		NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Shigella dysenteriae																	
IG864 ,	6	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG865 ,	7	+++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG866 ,	8a	+++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG867 ,	9a	++++	++++	++++	+++	+/-	++	-	-	-	-	+/-	-	-	-	++++	++++
IG943 ,	9	++++	++++	+++	+++	+/-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG944 ,	9	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG868 ,	10	-	-	+++	+++	+/-	++	-	-	-	-	+/-	-	-	-	++++	++++
IG706		++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++

TABLE 3 : HYBRIDIZATION RESULTS FOR SHIGELLA FLEXNERI SEROTYPES

Genus species Strain ID, serotype		NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Shigella flexneri																	
IG879 ,	1a	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	+++
IG945 ,	1a	-	-	-	-	-	-	-	+++	++++	++++	+++	+++	-	-	++++	+++
IG946 ,	1b	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	+++
IG878 ,	1b	-	-	-	-	-	-	-	++++	+++	+++	+++	++	-	-	++++	++
IG818 ,	1b	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	+++
RF973 ,	2a	-	-	-	-	-	-	-	++++	++++	++++	+++	++++	-	-	++++	++++
IG716 ,	2b	+	-	-	-	-	-	-	++++	++++	++++	+++	++++	-	-	++++	++++
IG877 ,	3a	++	++	-	-	-	-	-	++++	++++	++++	++++	++++	-	-	++++	+++
IG947 ,	3a	-	-	+++	-	++	++	+++	++++	++++	++++	+++	+++	-	-	++++	+++
IG876 ,	3b	-	-	+++	-	++	++	+++	++++	++++	++++	+++	++++	-	-	++++	+++
IG875 ,	3c	-	-	++++	-	+	++	+++	++++	++++	++++	++++	+++	-	-	++++	+++
IG825 ,	3	++++	++	-	-	-	-	-	++++	++++	++++	++++	++++	-	-	++++	++++
IG874 ,	4a	-	-	-	-	-	-	-	++++	++++	++++	+++	++++	-	-	++++	+++
IG948 ,	4a	-	-	-	-	-	-	-	+++	++++	++++	++++	+++	-	-	++++	+++
IG873 ,	4b	-	-	-	-	-	-	-	++++	++++	++++	+++	++++	-	-	++++	++++
IG838 ,	4	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	+++
IG872 ,	5	-	-	-	-	-	-	-	-	-	-	+++	++++	-	-	++++	++++

TABLE 3 : CONT'D

Genus species Strain ID, serotype		NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Shigella flexneri																	
IG949 ,	5	++	++	-	-	-	-	-	++++	++++	++++	++++	++++	-	-	++++	+++
IG823 ,	6	++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG871 ,	6	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG950 ,	6	+++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
RF951		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
RF944		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
RF947		-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	+++
RF947		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
RF950		-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	+++
IG763		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG764		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG765		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG766		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG767		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG768		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG770		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG771		++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++

TABLE 3 : CONT'D

Genus species Strain ID, serotype	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	 1706	CR3 437	 1864
Shigella flexneri																
IG772	++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG773	++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG775	-	-	-	-	-	-	-	++++	++++	++++	++++	+++	-	-	++++	++++
IG777	++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG778	++	++	++++	+	-	++	+++	++++	++++	++++	++++	++++	-	-	++++	+++
IG782	-	-	-	-	-	-	-	+++	++++	++++	+++	+++	-	-	++++	++++
IG724	-	-	++++	-	++	++	+++	+++	++++	++++	+++	+++	-	-	++++	+++
IG743	-	-	++++	-	-	++	+++	+++	++++	++++	+++	+++	-	-	++++	+++
IG744	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	++++
IG737	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	++++
IG735	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	++++
IG738	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	++++
IG736	-	-	+++	-	-	++	+++	++++	++++	++++	+++	+++	-	-	++++	+++
IG741	-	-	-	-	-	-	-	-	-	-	+++	++	-	-	++++	+++
IG740	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	++++
IG739	-	-	-	-	-	-	-	++++	++++	++++	+++	+++	-	-	++++	+++
IG742	-	-	++++	-	++	++	+++	+++	++++	++++	+++	+++	-	-	++++	+++

TABLE 3 : CONT'D

Genus species Strain ID, serotype	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Shigella flexneri																
IG817	-	-	-	-	-	-	-	+++	++++	++++	+++	+++	-	-	++++	+++
IG820	-	-	-	-	-	-	-	++++	++++	++++	+++	++	-	-	++++	+++
IG822	-	-	-	-	-	-	-	+++	++++	++++	+++	+++	-	-	++++	+++
IG709	-	-	-	-	-	-	-	-	-	-	+++	++++	-	-	++++	++++
IG711	-	-	-	-	-	-	-	++++	-	++++	+++	++++	-	-	++++	++++
IG726	++	++	++++	++	+/-	+++	+++	++++	++++	++++	++++	++++	-	-	++++	++++

TABLE 4 : HYBRIDIZATION RESULTS FOR SHIGELLA BOYDII SEROTYPES

Genus species Strain ID, serotype		NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Shigella boydii																	
IG832 ,	1	+++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
RF971 ,	2	++++	++++	-	ND	ND	ND	ND	-	ND	ND	+/-	ND	ND	ND	++++	++++
IG880 ,	3	++++	++++	-	-	-	-	-	-	-	-	-	-	-	-	++++	+++
IG935 ,	4	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG882 ,	5	-	-	+++	+++	++++	++	+++	-	-	-	++++	++++	++++	++++	++++	++++
IG936 ,	5	-	-	+++	+++	++++	++	+++	-	-	-	++++	+++	++++	++++	++++	+++
IG883 ,	6	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG884 ,	7	+	-	++++	+++	++++	++	+++	-	-	-	++++	++++	++++	++++	++++	-/+
IG885 ,	8	++++	++++	-	-	-	-	-	-	-	-	+	-	-	-	++++	++++
IG829 ,	9	-	-	+++	+++	++++	++	-	-	-	-	+++	+++	++++	++++	++++	+++
IG886 ,	9	-	-	-	-	-	-	-	-	-	-	+++	++++	++++	++++	++++	+/-
IG887 ,	10	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG937 ,	10	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG3231,	10	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG938 ,	11	-	-	++++	+++	++++	++	+++	-	-	-	+++	++++	++++	++++	++++	+++
IG888 ,	11	-	-	++++	+++	++++	++	+++	-	-	-	++++	++++	++++	++++	++++	+/-
IG889 ,	12	+	-	++++	+++	++++	-	-	-	-	-	+/-	-	++++	++++	++++	+++

TABLE 4 : CONT'D

Genus species Strain ID, serotype	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Shigella boydii																
RF974 , 13	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-/+
IG890 , 13	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
IG891 , 14	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG939 , 14	+++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG892 , 15	-	-	-	-	-	-	-	-	-	-	+/-	-	++++	++++	++++	+/-
IG700 , 16	-	-	+++	+++	++++	++	+++	-	-	-	++++	++++	++++	++++	++++	+++
IG701 , 17	-	-	++++	+	+++	+/-	++++	-	-	-	++++	++++	++	+++	++++	+++
IG702 , 18	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
RF948	++++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	++++
IG718	+++	++++	-	-	-	-	-	-	-	-	+/-	-	-	-	++++	+++

TABLE 5 : HYBRIDIZATION RESULTS FOR SHIGELLA SONNEI SEROTYPES

Genus species Strain ID	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
S.sonnei																
IG827	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG821	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG869	++++	++++	+++	+++	+++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG870	++++	++++	+++	++	+++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG929	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG930	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG931	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG932	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG933	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG934	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG951	++++	++++	++++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG952	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG953	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG954	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG955	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG956	++++	++++	++++	++++	++++	+++	++++	-	-	-	+++	++++	-	-	++++	++++
IG957	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++

TABLE 5 : CONT'D

Genus species Strain ID	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
S.sonnei																
IG958	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG959	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG960	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG961	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG962	++++	++++	+++	+++	++++	++	+++	-	-	-	++++	++++	-	-	++++	++++
IG963	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG964	++++	++++	+++	++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG965	++++	++++	++++	++++	++++	+++	++++	-	-	-	+++	++++	-	-	++++	++++
RF968	++++	++++	+++	+++	+++	++	+++	-	-	-	+++	+++	-	-	++++	++++
RF943	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
RF949	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG781	++++	++++	++++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG723	++++	++++	++++	++++	++++	+++	++++	-	-	-	+/-	-	-	-	++++	++++
IG717	++++	++++	++++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG713	++++	++++	++++	+	+++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG714	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG720	+++	+++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++

TABLE 5 : CONT'D

Genus species Strain ID	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
S.sonnei																
IG719	++++	++++	++++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG721	++++	++++	++++	+++	++++	+++	+++	-	-	-	+/-	-	-	-	++++	++++
IG712	++++	++++	++++	+	+++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG707	++++	++++	+++	+++	+++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG708	++++	++++	++++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG715	++++	++++	+++	+++	++++	+++	+++	-	-	-	+/-	-	-	-	++++	++++
IG831	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG830	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG731	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG730	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG733	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG728	++++	++++	++++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG732	++++	++++	+++	+++	++++	+++	+++	-	-	-	+++	++++	-	-	++++	++++
IG729	++++	++++	+++	+++	++++	+++	+++	-	-	-	+/-	-	-	-	++++	++++
IG734	++++	++++	+++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG727	++++	++++	++++	+++	++++	++	+++	-	-	-	+/-	-	-	-	++++	++++
IG966	++++	++++	++++	++	+++	++	+++	-	-	-	+++	+++	-	-	++++	++++

TABLE 5 : CONT'D

Genus species Strain ID	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
S.sonnei																
IG967	++++	++++	+++	++	+++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG968	++++	++++	++++	+++	++++	+++	++++	-	-	-	+++	++++	-	-	++++	++++
IG969	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG970	++++	++++	++++	++++	++++	+++	++++	-	-	-	+++	+++	-	-	++++	++++
IG971	++++	++++	++++	++++	++++	+++	++++	-	-	-	+++	++++	-	-	++++	++++
IG972	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG974	++++	++++	++++	++++	++++	+++	++++	-	-	-	+++	++++	-	-	++++	++++
IG975	++++	++++	+++	++	+++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG976	++++	++++	++++	++++	++++	+++	++++	-	-	-	+++	+++	-	-	++++	++++
IG979	++++	++++	+++	+++	+++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG980A	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	+++	-	-	++++	++++
IG980B	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++
IG982	++++	++++	+++	+++	++++	++	+++	-	-	-	+++	++++	-	-	++++	++++

TABLE 6A : HYBRIDIZATION OF SHIGELLA PROBES TO E. COLI

Genus species	Strain ID	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Enteroinvasive E. coli								
"	3138	++++	++++	-	-	-	-	-
"	3145	++++	++++	+++	+	+/-	+++	++++
"	3146	++	++++	-	-	-	-	-
"	3157	++++	++++	-	-	-	-	-
"	3037	++++	++++	-	-	-	-	-
Enterotoxigenic E. coli								
"	3118	-	-	-	-	-	-	-
"	3119	-	-	-	-	-	-	-
"	3120	-	-	-	-	-	-	-
"	3123	-	-	-	-	-	-	-
"	3127	-	-	-	-	-	-	-
"	3129	-	-	-	-	-	-	-
"	3132	-	-	-	-	-	-	-
"	3134	-	-	-	-	-	-	-
"	3136	+	-	-	-	-	-	-
"	3142	-	-	-	-	-	-	-
"	3147	-	-	-	-	-	-	-
"	3151	-	-	-	-	-	-	-
"	3154	-	-	-	ND	-	-	-
"	3156	-	-	-	-	-	-	-
"	3158	+	-	-	-	-	-	-
"	3160	-	-	-	-	-	-	-

TABLE 6A : CONT'D

Genus species	Strain ID	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Enteropathogenic E. coli - 0157 serotype								
"	3137	-	-	-	-	-	-	-
"	3140	-	-	-	-	-	-	-
"	3143	-	-	-	-	-	-	-
"	3144	-	-	-	-	-	-	-
"	3152	-	-	-	-	-	-	-
"	3155	-	-	-	-	-	-	-
"	3162	-	-	-	-	-	-	-
"	3163	-	-	-	-	-	-	-
"	3164	-	-	-	-	-	-	-
"	3165	-	-	-	-	-	-	-
"	3040	-	-	-	-	-	-	-
Enteropathogenic E. coli - non 0157 serotypes								
"	3038	+	-	+++	+	+++	++	+++
"	3039	+	-	-	-	-	-	-
"	3041	+	-	-	-	-	-	-
"	3042	-	-	-	-	-	-	-
"	3043	-	-	-	-	-	-	-
"	839	-	-	-	-	-	-	-
"	840	+	-	-	-	-	-	-
"	841	-	-	-	-	-	-	-
"	842	-	-	-	-	-	-	-
"	843	-	-	+++	-	-	++	+++
"	844	-	-	-	-	-	-	-
"	845	-	-	-	-	-	-	-

TABLE 6A : CONT'D

Genus species	Strain ID	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Enteropathogenic E. coli non - 0157 serotype								
"	846	++++	-	-	-	-	-	-
"	847	-	-	-	-	-	-	-
"	848	-	-	-	-	-	-	-
"	3149	-	-	-	-	-	-	-
Non-pathogenic E. coli								
"	3116	-	-	-	-	-	-	-
"	3117	-	-	-	-	-	-	-
"	3121	-	-	-	-	-	-	-
"	3122	-	-	-	-	-	-	-
"	3124	-	-	-	-	-	-	-
"	3125	-	-	-	-	-	-	-
"	3126	-	-	-	-	-	-	-
"	3128	-	-	-	-	-	-	-
"	3130	-	-	-	-	-	-	-
"	3131	-	-	-	-	-	-	-
"	3133	-	-	-	-	-	-	-
"	3135	-	-	-	-	-	-	-
"	3139	-	-	-	-	-	-	-
"	3148	-	-	-	-	-	-	-
"	3150	-	-	-	-	-	-	-
"	3153	-	-	-	-	-	-	-
"	3158	-	-	-	-	-	-	-
"	3161	-	-	-	-	-	-	-

TABLE 6B : HYBRIDIZATION OF SHIGELLA PROBES TO E. COLI

Genus species	Strain ID	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Enteroinvasive E. coli										
"	3138	-	-	-	+/-	-	-	-	-	++++
"	3145	-	-	-	+/-	-	-	-	+	+++
"	3146	-	-	-	++++	++++	-	-	+	+++
"	3157	-	-	-	+/-	-	-	-	++	+++
"	3037	-	-	-	+/-	-	-	-	++	-/+
Enterotoxigenic E. coli										
"	3118	-	-	-	+/-	-	-	-	-	-
"	3119	-	-	-	+/-	-	-	-	-	-
"	3120	-	-	-	+/-	-	-	-/+	-	-/+
"	3123	-	-	-	+/-	-	-	-/+	-	-
"	3127	-	-	-	+/-	-	-	-/+	-	-
"	3129	-	-	-	+/-	-	-	-/+	++	-/+
"	3132	-	-	-	+/-	-	-	-	+	-
"	3134	-	-	-	+/-	-	-	-	-	-
"	3136	-	-	-	+/-	-	-	-	+	-
"	3142	-	-	-	+/-	-	-	-	-	-
"	3147	-	-	-	+/-	-	-	-/+	-	-
"	3151	-	-	-	+/-	-	-	-	-	-
"	3154	-	ND	ND	+/-	-	-	-	-	-
"	3156	-	-	-	+/-	-	-	-	++	-/+
"	3158	-	-	-	+	-	-	-	++	ND
"	3160	-	-	-	+/-	-	-	-	+	-

TABLE 6B : CONT'D

Genus species	Strain ID	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Enteropathogenic E. coli - O157 serotype										
"	3137	-	-	-	+/-	-	-	-	-	-
"	3140	-	-	-	+/-	-	-	-	-	-
"	3143	-	-	-	+/-	-	-	-	-	-
"	3144	-	-	-	+/-	-	-	-	-	-
"	3152	-	-	-	+/-	-	-	-	-	-
"	3155	-	-	ND	+/-	-	-	-	-	-
"	3162	-	-	-	+/-	-	-	-	-	-
"	3163	-	-	-	+/-	-	-	-	-	-
"	3164	-	-	-	+/-	-	-	-	-	-
"	3165	-	-	-	+/-	-	-	-	-	-
"	3040	-	-	-	+/-	-	-	-	-	-
Enteropathogenic E. coli - non O157 serotypes										
"	3038	-	-	-	+/-	-	-	-	-	-
"	3039	+++	++++	++++	+/-	-	-	-	+	-
"	3041	-	-	-	+	-	-	-	++	ND
"	3042	-	-	-	+/-	-	-	-	-	-
"	3043	-	-	-	+/-	-	-	-	-	-
"	839	-	-	-	+/-	-	-	-	-	-
"	840	-	-	-	+/-	-	-	-	++	-
"	841	-	-	-	+/-	-	-	-	-	-
"	842	-	-	-	+/-	-	-	-/+	-	-
"	843	-	-	-	+/-	-	-	-	-	-
"	844	-	-	-	+/-	-	-	-	-	-/+
"	845	-	-	-	+/-	-	-	-	+	-

TABLE 6B : CONT'D

Genus species	Strain ID	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Enteropathogenic E. coli non - O157 serotype										
"	846	-	-	-	+/-	-	-	-	-	-
"	847	-	-	-	+/-	-	-	-	-	-
"	848	-	-	-	+/-	-	-	-	-	-
"	3149	-	-	-	+/-	-	-	-	-	-
Non-pathogenic E. coli										
"	3116	-	-	-	+/-	-	-	-	++	-
"	3117	-	-	-	+/-	-	-	-	-	-
"	3121	-	-	-	+/-	-	-	-	-	-
"	3122	-	-	-	+/-	-	-	-	-	-
"	3124	-	-	-	+/-	-	-	-	-	-
"	3125	-	-	-	+/-	-	-	-	-	-
"	3126	-	-	-	+/-	-	-	-	-	-
"	3128	-	-	-	+/-	-	-	-	+	-/+
"	3130	-	-	-	+/-	-	-	-	-	-
"	3131	-	-	-	+/-	-	-	-/+	-	-
"	3133	-	-	-	+/-	-	-	-	-	-
"	3135	-	-	-	+/-	-	-	-	-	-
"	3139	-	-	-	+/-	-	-	-/+	-	-
"	3148	-	-	-	+/-	-	-	-	-	-
"	3150	-	-	-	+/-	-	-	-	+	-
"	3153	-	-	-	+/-	-	-	-/+	-	-
"	3158	-	ND	ND	+/-	-	-	-	+	ND
"	3161	-	-	-	+/-	-	-	-/+	+	ND

TABLE 7A : HYBRIDIZATION OF SHIGELLA PROBES TO MISC. ENTEROBACTERIACEAE

Genus species	GT#	ATCC#	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Alteromonas putrefaciens	1495	8071	-	-	-	-	-	-	-
Acinetobacter calcoaceticus	1972		-	-	-	ND	ND	ND	ND
Citrobacter diversus	1608		-	-	-	-	-	-	-
" diversus	1475	27156	-	-	-	-	-	-	-
" amalonaticus	1607		-	-	-	-	-	-	-
" amalonaticus	0689	25406	-	-	-	-	-	-	-
" amalonaticus	0690	25405	-	-	-	-	-	-	-
" freundii	0041		-	-	-	-	-	-	-
" freundii	0031		-	-	-	-	-	-	-
" freundii	1597		-	-	-	-	-	-	-
" freundii	1476	29935	-	-	-	-	-	-	-
" freundii	1477	33128	-	-	-	-	-	-	-
" freundii	1491	8090	-	-	-	-	-	-	-
" freundii	1591		-	-	-	-	-	-	-
" freundii	1595		-	-	-	-	-	-	-
" freundii	1599		-	-	-	-	-	-	-
" freundii	0685		-	-	-	-	-	-	-
" freundii	3241		-	-	-	-	-	-	-

TABLE 7A : CONT'D

Genus species	GT#	ATCC#	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Citrobacter freundii	0038		-	-	-	-	-	-	-
" freundii	0036		-	-	-	ND	ND	ND	ND
" freundii	0035		-	-	-	-	-	-	-
" freundii	0034		-	-	-	-	-	-	-
" freundii	0033		-	-	-	-	-	-	-
" freundii	0032		-	-	-	-	-	-	-
" freundii	0037		-	-	-	-	-	-	-
" freundii	0039		-	-	-	ND	-	-	-
" freundii	0040		-	-	-	-	-	-	-
Enterobacter aerogenes	1487	29940	-	-	-	-	-	-	-
" aerogenes	0047	13048	-	-	-	-	-	-	-
" agglomerans	0048		-	-	-	-	-	-	-
" agglomerans	0049		-	-	-	-	-	-	-
" agglomerans	1467	29917	-	-	-	-	-	-	-
" agglomerans	1468	29918	-	-	-	-	-	-	-
" agglomerans	1469	29919	-	-	-	-	-	-	-
" agglomerans	1470	29920	-	-	-	-	-	-	-
" agglomerans	1471	29921	-	-	-	-	-	-	-

TABLE 7A : CONT'D

Genus species	GT#	ATCC#	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Enterobacter agglomerans	1472	29922	-	-	-	-	-	-	-
" agglomerans	1473	29923	-	-	-	-	-	-	-
" agglomerans	1488	29904	-	-	-	-	-	-	-
" agglomerans	1489	29915	-	-	-	-	-	-	-
" agglomerans	1490	29916	-	-	-	-	-	-	-
" agglomerans	1474	27998	-	-	-	-	-	-	-
" amnigenus	1482	33072	-	-	-	-	-	-	-
" cloacae	0052		-	-	-	-	-	-	-
" cloacae	3042		-	-	-	-	-	-	-
" cloacae	0050		-	-	-	-	-	-	-
" cloacae	3041		-	-	-	-	-	-	-
" cloacae	1159		-	-	-	-	-	-	-
" cloacae	1337		-	-	-	-	-	-	-
" cloacae	1481	29941	-	-	-	-	-	-	-
" cloacae	1492	13047	-	-	-	-	-	-	-
" cloacae	3043		-	-	-	-	-	-	-
" gergoviae	1486	33028	-	-	-	-	-	-	-
" intermedium	0677	33110	-	-	-	-	-	-	-

TABLE 7A : CONT'D

Genus species	GT#	ATCC#	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Enterobacter sakazakii	0063		-	-	-	-	-	-	-
" sakazakii	1483	29544	-	-	-	-	-	-	-
" taylorae	1497	35317	-	-	-	-	-	-	-
" taylorae	0065		-	-	-	-	-	-	-
Escherichia blattae	1460		-	-	-	-	-	-	-
" fergusonii	1453		-	-	-	-	-	-	-
" fergusonii	1459		-	-	-	-	-	-	-
" hermanii	1216	33650	-	-	-	-	-	-	-
" vulneri	1456		-	-	-	-	-	-	-
" vulneri	1217	33821	-	-	-	-	-	-	-
Hafnia alvei	0241	29927	-	-	-	-	-	-	-
" alvei	1153		-	-	-	-	-	-	-
Klebsiella oxytoca	1606		-	-	-	-	-	-	-
" oxytoca	1605		-	-	-	-	-	-	-
" oxytoca	1503	13182	-	-	-	-	-	-	-
" ozaenae	1499	11296	-	-	-	-	-	-	-
" planticola	1478	33531	-	-	-	-	-	-	-
" pneumoniae	1150		-	-	-	-	-	-	-

TABLE 7A : CONT'D

Genus species	GT#	ATCC#	NT 6	1911 1500 1501	NT 11-2	1682	1683	1708	1709
Klebsiella pneumoniae	1500	13883	+	-	-	-	-	-	-
" pneumoniae	1502	29939	-	-	-	-	-	-	-
" pneumoniae	0252		-	-	-	-	-	-	-
" pneumoniae	1177		-	-	-	-	-	-	-
" terrigena	1479	33257	-	-	-	-	-	-	-
Morganella morganii	1147		-	-	-	-	-	-	-
Proteus mirabilis	1148		-	-	-	ND	-	-	ND
" mirabilis	1208		-	-	-	-	-	-	-
" mirabilis	1493	25933	-	-	-	ND	ND	ND	ND
" mirabilis	1496	29906	-	-	-	-	-	-	-
" mirabilis	1501	7002	-	-	-	-	-	-	-
" vulgaris	0370		-	-	-	-	-	-	-
Providencia stuartii	3044		-	-	-	-	-	-	-
Psuedomonas aeruginosa	3045		-	-	-	-	-	-	-
Salmonella typhimurium	0389	23566	-	-	-	ND	ND	ND	ND
Serratia marcescens	0392	29937	-	-	ND	-	-	-	-
" marcescens	1151		-	-	-	-	-	-	-
Yersinia enterocolitica	0424		-	-	-	-	-	-	-
" enterocolitica	3219		-	-	-	-	-	-	-

TABLE 7B : HYBRIDIZATION OF SHIGELLA PROBES TO MISC. ENTEROBACTERIACEAE

Genus species	GT#	ATCC#	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1364
Alteromonas putrefaciens	1495	8071	-	-	-	+/-	-	-	-	-	-
Acinetobacter calcoaceticus	1972		-	ND	ND	+/-	ND	ND	ND	-	-
Citrobacter diversus	1608		-	-	-	+/-	-	-	-/+	-	-
" diversus	1475	27156	-	-	-	+/-	-	-	-/+	-	-
" amalonaticus	1607		-	-	-	+/-	-	-	-/+	-	-
" amalonaticus	0689	25406	-	-	-	+/-	-	-	-/+	-	-
" amalonaticus	0690	25405	-	-	-	+/-	-	-	-/+	-	-
" freundii	0041		-	-	-	+/-	-	-	-	-	-
" freundii	0031		-	-	-	+/-	-	-	-	-	-
" freundii	1597		-	ND	ND	+/-	-	-	-	-	-
" freundii	1476	29935	-	-	-	+/-	-	-	-	-	-
" freundii	1477	33128	-	-	-	+/-	-	-	-	-	-
" freundii	1491	8090	-	-	-	+/-	-	-	-	-	-
" freundii	1591		-	-	-	+/-	-	-	-	-	-
" freundii	1595		-	-	-	+/-	-	-	-	-	-
" freundii	1599		-	-	-	+/-	-	-	-	-	-
" freundii	0685		-	-	-	+/-	-	-	-	-	-
" freundii	3241		-	-	-	+/-	-	-	-/+	-	-

TABLE 7B : CONT'D

Genus species	GT#	ATCC#	NT 18-1a	1712	1713	NT 19-2	1684	ompA 1707	ompA 1706	CR3 437	CR3 1864
Citrobacter freundii	0038		-	-	-	+/-	-	-	-	ND	-
" freundii	0036		-	ND	ND	+/-	ND	ND	ND	ND	-
" freundii	0035		-	-	-	+/-	-	-	-	ND	-
" freundii	0034		-	-	-	+/-	-	-	-	ND	-
" freundii	0033		-	-	-	+/-	-	-	-	ND	-
" freundii	0032		-	-	-	+/-	-	-	-	ND	-
" freundii	0037		-	-	-	+/-	-	-	-	ND	-
" freundii	0039		-	ND	ND	+/-	-	-	-	ND	-
" freundii	0040		-	-	-	+/-	-	-	-	ND	-
Enterobacter aerogenes	1487	29940	-	-	-	+/-	-	-	-	-	-
" aerogenes	0047	13048	-	-	-	+/-	-	-	-	-	-
" agglomerans	0048		-	-	-	+/-	-	-	-	-	-
" agglomerans	0049		-	-	-	+/-	-	-	-	-	-
" agglomerans	1467	29917	-	-	-	+/-	-	-	-	-	-
" agglomerans	1468	29918	-	-	-	+/-	-	-	-	-	-
" agglomerans	1469	29919	-	-	-	+/-	-	-	-	-	-
" agglomerans	1470	29920	-	-	-	+/-	-	-	-	-	-
" agglomerans	1471	29921	-	-	-	+/-	-	-	-	-	-

TABLE 7B : CONT'D

Genus species		GT#	ATCC#	NT 1712 1713 18-1a			NT 1684 19-2	1685	ompA 1707	1706	CR3 437	1864
Enterobacter	agglomerans	1472	29922	-	-	-	+/-	-	-	-	-	-
"	agglomerans	1473	29923	-	-	-	+/-	-	-	-	-	-
"	agglomerans	1488	29904	-	-	-	+/-	-	-	-	-	-
"	agglomerans	1489	29915	-	-	-	+/-	-	-	+/-	-	-
"	agglomerans	1490	29916	-	-	-	+/-	-	-	-	-	-
"	agglomerans	1474	27998	-	-	-	+/-	-	-	-	-	-
"	amnigenus	1482	33072	-	-	-	+/-	-	-	-	-	-
"	cloacae	0052		-	-	-	+/-	-	-	+	-	-
"	cloacae	3042		-	-	-	+/-	-	-	-	-	-
"	cloacae	0050		-	-	-	+/-	-	-	-	-	-
"	cloacae	3041		-	-	-	+/-	-	-	-	-	-
"	cloacae	1159		-	-	-	+/-	-	-	-	-	-
"	cloacae	1337		-	-	-	+/-	-	-	-	-	-
"	cloacae	1481	29941	-	-	-	+/-	-	-	-	-	-
"	cloacae	1492	13047	-	-	-	+/-	-	-	-	-	-
"	cloacae	3043		-	-	-	+/-	-	-	-	-	-
"	gergoviae	1486	33028	-	-	-	+/-	-	-	-	-	-
"	intermedium	0677	33110	-	-	-	+/-	-	-	-	-	-

TABLE 7B : CONT'D

Genus species	GT#	ATCC#	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Enterobacter sakazakii	0063		-	-	-	+/-	-	-	-	-	-
" sakazakii	1483	29544	-	-	-	+/-	-	-	-	-	-
" taylorae	1497	35317	-	-	-	+/-	-	-	-	-	-
" taylorae	0065		-	-	-	+/-	-	-	-	-	-
Escherichia blattae	1460		-	-	-	+/-	-	-	++++	-	-
" fergusonii	1453		-	-	-	+/-	-	+	+	-	-
" fergusonii	1459		-	-	-	+/-	-	++	+++	-	-
" hermanii	1216	33650	-	ND	ND	+/-	-	-	-	-	-
" vulneri	1456		-	-	-	+/-	-	-	-	-	-
" vulneri	1217	33821	-	-	-	+/-	-	-	-	-	-
Hafnia alvei	0241	29927	-	-	-	+/-	-	-	-	-	-
" alvei	1153		-	-	-	+/-	-	-	-	-	-
Klebsiella oxytoca	1606		-	-	-	+/-	-	-	-	ND	-
" oxytoca	1605		-	-	ND	+/-	-	-	-	ND	-
" oxytoca	1503	13182	-	-	-	+/-	-	-	-	-	-
" ozaenae	1499	11296	-	-	-	+/-	-	-	-	-	-
" planticola	1478	33531	-	-	-	+/-	-	-	-	-	-
" pneumoniae	1150		-	-	-	+/-	-	-	-	-	-

TABLE 7B : CONT'D

Genus species	GT#	ATCC#	NT 18-1a	1712	1713	NT 19-2	1684 1685	ompA 1707	ompA 1706	CR3 437	CR3 1864
Klebsiella pneumoniae	1500	13883	-	-	-	+/-	-	-	-	-	-
" pneumoniae	1502	29939	-	-	-	+/-	-	-	-	-	-
" pneumoniae	0252		-	-	-	+/-	-	-	-	-	-
" pneumoniae	1177		-	-	-	+/-	-	-	-	-	-
" terrigena	1479	33257	-	-	-	+/-	-	-	-	-	-
Morganella morganii	1147		-	-	-	+/-	-	-	-	-	-
Proteus mirabilis	1148		-	ND	ND	+/-	-	-	-	-	-
" mirabilis	1208		-	ND	ND	+/-	-	-	-	-	-
" mirabilis	1493	25933	-	ND	ND	+/-	ND	ND	ND	-	-
" mirabilis	1496	29906	-	-	-	+/-	-	-	-	-	-
" mirabilis	1501	7002	-	-	-	+/-	-	-	-	-	-
" vulgaris	0370		-	-	-	+/-	-	-	-	-	-
Providencia stuartii	3044		-	-	-	+/-	-	-	-	-	-/+
Psuedomonas aeruginosa	3045		-	-	-	+/-	-	-	-	-	-
Salmonella typhimurium	0389	23566	-	ND	ND	+/-	ND	ND	ND	-	-
Serratia marcescens	0392	29937	-	-	-	+/-	-	-	-	-	-
" marcescens	1151		-	ND	-	+/-	-	-	-	-	-
Yersinia enterocolitica	0424		-	-	-	+/-	-	-	-	-	-
" enterocolitica	3219		-	-	-	+/-	-	-	-	-	-

TABLE 8 : HYBRIDIZATION OF SHIGELLA PROBES TO BACTERIA COMMONLY FOUND IN STOOL

Genus/Species	GT#	ATCC#	NT 6			NT 19-2		ompA		CR3
			1500	1501	1911	1684	1685	1706	1707	1864
Acinetobacter calcoaceticus	0002	19606	-	-/+	-	-	-	-	-	-
Acinetobacter lwoffii	0004	9957	-	-	-	-	-	-	-	-
Aeromonas hydrophila	0006	7965	-	-/+	-	-	-	-	-	-
Aeromonas sobria	0007	9071	-	-	-	-	-	-	-	-
Alteromonas putrefaciens	1495	8071	-	-	-	-	-	-	-	-
Citrobacter amalonaticus	0690	25405	-	-	-	-	-	-	-	-
Edwardsiella tarda	0569	15947	-	-	-	-	-	-	-	-
Haemophilus influenzae	0244	19418	-	+/-	-	-	-	+/-	-	-
Plesiomonas shigelloides	2197	14029	-	-	-	-	-	-	-	-
Providencia alcalifaciens	0371	9886	-	-/+	-	-	-	-	-	-
Providencia rettgeri	0373	9944	-	-	-	-	-	-	-	-
Providencia stuartii	0375	29914	-	-	-	-	-	-	-	-
Salmonella arizona	0799	13314	-	-	-	-	-	-	-	-
Salmonella typhimurium	0389	23566	-	+/-	-	-	-	-	-	-
Vibrio parahemolyticus	0568	17802	-	-	-	-	-	-	-	-
Xanthomonas maltophila	0417	13637	-	-	-	-	-	-	-	-
Yersinia enterocolitica	0419	9610	-	-	-	-	-	-	-	-
Yersinia pseudotuberculosis	0519	29833	-	-	-	-	-	-	-	-

TABLE 8 : CONT'D

Genus/Species	GT#	ATCC#	NT 6			NT 19-2		ompA		CR3
			1500	1501	1911	1684	1685	1706	1707	1864
Yersinia ruckeri	0522	29473	-	+/-	-	-	-	-	-	-
Alcaligenes denitrificans ss xyloxidans	0001	27062	-	-/+	-	-	-	-	-	-
Alcaligenes faecalis	0610	875	-	-	-	-	-	-	-	-
Kingella kingae	0247	23330	-	-	-	-	-	-	-	-
Kingella dendrificans	0245	33394	-	-	-	-	-	-	-	-
Kingella indologenes	0246	25869	-	-	-	-	-	-	-	-
Moraxella osloensis	0301	19962	-	-	-	-	-	-	-	-
Neisseria cinerea	0307	14685	-	-	-	-	-	-	-	-
Neisseria flavescens	0310	13120	-	-/+	-	-	-	-	-	-
Neisseria gonorrhoeae, type	0315	19424	-	-/+	-	-	-	-	-	-
Neisseria meningitidis	0348		-	-/+	-	-	-	-	-	-
Neisseria mucosa	0353	19696	-	-	-	-	-	-	-	-
Pseudomonas acidovorans, type	0376	15668	-	-	-	-	-	-	-	-
Achromobacter xerosis	0810	14780	-	-	-	-	-	-	-	-
Gardnerella vaginalis	0240	1408	-	-	-	-	-	-	-	-
Acidaminococcus fermantans	3129	25085	-	-	-	-	-	-	-	-
Ruminococcus bromeii	3142	27255	-	-	-	-	-	-	-	-

TABLE 8 : CONT'D

Genus/Species	GT#	ATCC#	NT 6			NT 19-2		ompA		CR3
			1500	1501	1911	1684	1685	1706	1707	1864
Bacteroides gracilis	0716	33236	-	-	-	-	-	-	-	-
Bacteroides ureolyticus	0715	33387	-	-/+	-	-	-	-	-	-
Campylobacter jejuni,type	0022	33560	-	-	-	-	-	-	-	-
Campylobacter coli	0016	33559	-	-/+	-	-	-	-	-	-
Campylobacter laridis	0024	35223	-	-	-	-	-	-	-	-
Wolinella curva	2224	35224	-	-	-	-	-	-	-	-
Wolinella recta	0718	33238	-	-	-	-	-	-	-	-
Wolinella succinogenes	0614	29543	-	-	-	-	-	-	-	-
Bacillus cereus	0008	14579	-	-	-	-	-	-	-	-
Butyrivibrio fibrosolvens	3139	19171	-	-	-	-	-	-	-	-
Clostridium difficile	0043	9689	-	-/+	-	-	-	-	-	-
Clostridium perfringens	0044	3624	-	-/+	-	-	-	-	-	-
Clostridium sordellii	0567	9714	-	-/+	-	-	-	-	-	-
Eubacterium lentum	2196	25559	-	-	-	-	-	-	-	-
Eubacterium rectale	0236	35183	-	-/+	-	-	-	-	-	-
Lactobacillus acidophilus	0256	4356	-	-	-	-	-	-	-	-
Lactobacillus casei	0805	393	-	-	-	-	-	-	-	-
Lactobacillus minutus	0257	33267	-	-/+	-	-	-	-	-	-

TABLE 8 : CONT'D

Genus/Species	GT#	ATCC#	NT 6 1500	NT 6 1501	NT 6 1911	NT 19-2 1684	NT 19-2 1685	ompA 1706	ompA 1707	CR3 1864
Lactobacillus plantarum	0258	8014	-	-	-	-	-	-	-	-
Listeria grayi	0674		-	+/-	-	-	-	-	-	-
Listeria innocua	0260		-	+/-	-	-	-	-	-	-
Listeria innocua	0750		-	-/+	-	-	-	-	-	-
Listeria ivanovii	1037		-	+/-	-	-	-	-	-	-
Listeria monocytogenes	1016		-	+/-	-	-	-	-	-	-
Listeria seeligeri	0287		-	-	-	-	-	-	-	-
Listeria welshrimpi	0291		-	+/-	-	-	-	-	-	-
Peptococcus asaccharolyticus	0360	29743	-	-	-	-	-	-	-	-
Peptococcus magnus	0361	29328	-	-	-	-	-	-	-	-
Peptostreptococcus anaerobius	0359	27337	-	-	-	-	-	-	-	-
Sarcina maxima	0391	33910	-	-/+	-	-	-	-	-	-
Staphylococcus aureus	0399	12600	-	-/+	-	-	-	-	-	-
Staphylococcus epidermidis	0401	14990	-	-/+	-	-	-	-	-	-
Streptococcus agalactiae	0405	13813	-	-/+	-	-	-	-	-	-
Streptococcus faecalis	0406	19433	-	-/+	-	-	-	-	-	-
Streptococcus faecium	0407	6056	-	-/+	-	-	-	-	-	-
Streptococcus mutans	0412	25175	-	-/+	-	-	-	-	-	-
Streptococcus salivarius	0410	13419	-	-/+	-	-	-	-	-	-

TABLE 8 : CONT'D

Genus/Species	GT#	ATCC#	NT 6 1500	NT 6 1501	NT 6 1911	NT 19-2 1684	NT 19-2 1685	ompA 1706	ompA 1707	CR3 1864
Streptococcus sanguis	0411	10556	-	+/-	-	-	-	-	-	-
Actinomyces israelii	0005	10049	-	-	-	-	-	-	-	-
Bifidobacterium dentium	0012	27534	-	-	-	-	-	-	-	-
Bifidobacterium bifidum	0571	35914	-	-	-	-	-	-	-	-
Corynebacterium genitalium	0045	33031	-	-	-	-	-	-	-	-
Mycobacterium smegmatis	0306	14468	-	-	-	-	-	-	-	-
Propionibacterium acnes	0363	6919	-	-	-	-	-	-	-	-
Fusobacterium mortiferum	0573	9817	-	-/+	-	-	-	-	-	-
Fusobacterium necrophorum	0238	25286	-	-	-	-	-	-	-	-
Mobiluncus mulieris	0300	35243	-	-	-	-	-	-	-	-
Veionella atypica	0413	14894	-	-	-	-	-	-	-	-
Bacteroides fragilis	0010	23745	-	-/+	-	-	-	-	-	-
Bacteroides thetaiotaomicron	0572	29741	-	-/+	-	-	-	-	-	-
Bacteroides melaninogenicus	0011	25845	-	-	-	-	-	-	-	-
Flavobacterium meningosepticum	0237	13253	-	-/+	-	-	-	-	-	-
Candida albicans	0028	18804	-	-	-	-	-	-	+/-	-
Candida glabrata	0029	2001	-	-	-	-	-	-	-	-
Candida stellatoidae	0609	36232	-	-	-	-	-	-	-	-
Candida tropicalis	0570	750	-	-	-	-	-	-	-	-

TABLE 9

LIST OF OLIGONUCLEOTIDE PROBES DESIGNED FROM SHIGELLA SPECIFIC FRAGMENTS

Parental DNA Fragment	Oligonucleotide Probe No. (SEQ ID NO)		Length of Probes (bases)	Sequence of oligonucleotide probes
NT 6 and adjacent 3' sequence (164 b)	1500	(SEQ ID NO:14)	35	5' TTGCAGCGCCTCTACTACCGGATACAGCCTCCATT 3'
	1501	(SEQ ID NO:15)	35	5' CCTCCTTCAGGGCGGATTCCAGCCGTTCACATTGT 3'
	1911	(SEQ ID NO:16)	40	5' CCGATCTTCTATTGTACGACGGTGTTCGTCAAAAGCTAAT 3'
NT 11-2 (796 b)	1682	(SEQ ID NO:17)	41	5' CTGGTGAACAACGTCTTACAAAGATGGTTCCTGGATGGATT 3'
	1683	(SEQ ID NO:18)	41	5' AGTCTTTCCGTGTTTCTCAGAAATGGGGGCAACGTGCAAAA 3'
	1708	(SEQ ID NO:19)	35	5' CCACCGTTGAAGCGTAAACCGTTTGACCGATGGAT 3'
	1709	(SEQ ID NO:20)	36	5' GCTGGGGTCTACAGGTGCAATAACCACTTAGACGGT 3'
NT 14 (786 b)	437	(SEQ ID NO:21)	48	5' CGATGATGCCATTCTCTGCCAGCTCCGTCTGGGAGCCGCCG-GGTTTCC 3'
NT 15 (587 b)	1864	(SEQ ID NO:22)	17	5' GGAGCAGTCTGGTCTGA 3'
NT18-1a (630 b)	1712	(SEQ ID NO:23)	37	5' CCTGTGGCTCTCGGTTCTGATGGTATAGCAACTAAAT 3'
	1713	(SEQ ID NO:24)	37	5' CAAGG[illegible]TTCGGAATTGAGTGGGGAGTTGCGAAAT 3'
NT19-2 (388 b)	1684	(SEQ ID NO:25)	35	5' CAGGCAATCGAAGCATATCGCGGTTCTCCACAACT 3'
	1685	(SEQ ID NO:26)	35	5' TGAATGCGCTGACCGAAAACCAGCGCTGGGTATCT 3'
ompA	1706	(SEQ ID NO:27)	35	5' GTGATGGCCCATTCAACACCACCTGCGAATACCGG 3'
	1707	(SEQ ID NO:28)	35	5' CTCAGATTCACCTGTCACATTGTTGTGAGCTTTGG 3'

Table 10

Summary of Probe Hybridization Results to Shigella Serotypes
(+ signal used as a cut-off for detectability)

SEROTYPE	Tested	NT-6	1911 1500 1501	NT11-2	1682	1683	1708	1709	NT18-1a	1712	1713	NT19-2	1684	1685	1707	1706	437	1864
S. Dysenteriae																		
1	8	-	-	-	-	-	-	-	6	6	6	-	-	-	8	8	8	7
2	2	1	1	2	2	2	-	-	-	-	-	-	-	-	2	2	2	2
3	3	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
4	3	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
5	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
6	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
7	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
8	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
9	3	3	3	2	2	-	1	-	-	-	-	-	-	-	-	-	3	3
10	1	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	1	1
untyped	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
	25																	
S. flexneri																		
1	5	-	-	-	-	-	-	-	5	5	5	5	5	5	-	-	5	5
2	2	1	-	-	-	-	-	-	2	2	2	2	2	2	-	-	2	2
3	5	2	2	3	-	3	3	3	5	5	5	5	5	5	-	-	5	5
4	4	-	-	-	-	-	-	-	4	4	4	4	4	4	-	-	4	4
5	2	1	1	-	-	-	-	-	1	1	1	2	2	2	-	-	2	2
6	3	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
untyped	36	16	16	20	16	2	20	20	34	33	34	36	36	36	-	-	36	36
57																		

Table 10 (cont')

SEROTYPE	Tested	NT-6	1911 1500 1501	NT11-2	1682	1683	1708	1709	NT18-1a	1712	1713	NT19-2	1684	1685	1707	1706	437	1864
S. boydii																		
1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
2	1	1	1	-	ND	ND	ND	ND	-	ND	ND	-	ND	ND	ND	ND	1	1
3	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
4	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
5	2	-	-	2	2	2	2	2	-	-	-	2	2	2	2	2	2	2
6	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
7	1	1	-	1	1	1	1	1	-	-	-	1	1	1	1	1	1	-
8	1	1	1	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1
9	2	-	-	1	1	1	1	-	-	-	-	2	2	2	2	2	2	1
10	3	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
11	2	-	-	2	2	2	2	2	-	-	-	2	2	2	2	2	2	1
12	1	1	-	1	1	1	-	-	-	-	-	-	-	-	1	1	1	1
13	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
15	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-
16	1	-	-	1	1	1	1	1	-	-	-	1	1	1	1	1	1	1
17	1	-	-	1	1	1	-	1	-	-	-	1	1	1	1	1	1	1
18	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
untyped	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
	27																	
S. sonnei																		
1	64	64	64	64	64	64	64	64	-	-	-	33	33	33	-	-	64	64

TABLE 11 : SUMMARY OF HYBRIDIZATION OF SHIGELLA SPECIFIC FRAGMENTS AND OLIGONUCLEOTIDE CAPTURE/DETECTION PROBES TO SHIGELLA SEROTYPES (++ SIGNAL USED AS CUT-OFF FOR DETECTABILITY).

SEROTYPE	# TESTED	NT6	1911 1500 1501	NT11 -2	1682 1683	1708 1709	NT18 -1a	1712 1713	NT19 -2	1684 1685	ompA 1706 1707	CR3 437c 1864
S. dysenteriae												
1	8	-	-	-	-	-	6	6	-	-	8	7
2	2	1	1	2	2	-	-	-	-	-	2	2
3	3	3	3	-	-	-	-	-	-	-	-	3
4	3	3	3	-	-	-	-	-	-	-	-	3
5	1	1	1	-	-	-	-	-	-	-	-	1
6	1	1	1	-	-	-	-	-	-	-	-	1
7	1	1	1	-	-	-	-	-	-	-	-	1
8	1	1	1	-	-	-	-	-	-	-	-	1
9	3	3	3	2	-	-	-	-	-	-	-	3
10	1	-	-	1	-	-	-	-	-	-	-	1
untyped	1	1	1	-	-	-	-	-	-	-	-	1
	25											

TABLE 11 : CONT'D

SEROTYPE	# TESTED	NT6	1911 1500 1501	NT11 -2	1682 1683	1708 1709	NT18 -1a	1712 1713	NT19 -2	1684 1685	ompA 1706 1707	CR3 437c 1864
S. flexneri												
1	5	-	-	-	-	-	5	5	5	5	-	5
2	2	-	-	-	-	-	2	2	2	2	-	2
3	5	2	2	3	-	3	5	5	5	5	-	5
4	4	-	-	-	-	-	4	4	4	4	-	4
5	2	1	1	-	-	-	1	1	2	2	-	2
6	3	3	3	-	-	-	-	-	-	-	-	3
untyped	36	16	16	20	-	20	34	33	36	36	-	36
	57											
S. boydii												
1	1	1	1	-	-	-	-	-	-	-	-	1
2	1	1	1	-	ND	ND	-	ND	-	ND	ND	1
3	1	1	1	-	-	-	-	-	-	-	-	1
4	1	1	1	-	-	-	-	-	-	-	-	1
5	2	-	-	2	2	2	-	-	2	2	2	2
6	1	1	1	-	-	-	-	-	-	-	-	1
7	1	-	-	1	1	1	-	-	1	1	1	-

TABLE 11 : CONT'D

SEROTYPE	# TESTED	NT6	1911 1500 1501	NT11 -2	1682 1683	1708 1709	NT18 -1a	1712 1713	NT19 -2	1684 1685	ompA 1706 1707	CR3 437c 1864
S. boydii												
8	1	1	1	-	-	-	-	-	-	-	-	1
9	2	-	-	1	1	-	-	-	2	2	2	1
10	3	3	3	-	-	-	-	-	-	-	-	3
11	2	-	-	2	2	2	-	-	2	2	2	1
12	1	-	-	1	1	-	-	-	-	-	1	1
13	2	-	-	-	-	-	-	-	-	-	-	-
14	2	2	2	-	-	-	-	-	-	-	-	2
15	1	-	-	-	-	-	-	-	-	-	1	-
16	1	-	-	1	1	1	-	-	1	1	1	1
17	1	-	-	1	-	-	-	-	1	1	1	1
18	1	1	1	-	-	-	-	-	-	-	-	1
untyped	2	2	2	-	-	-	-	-	-	-	-	2
	27											
S. sonnei												
1	64	64	64	64	64	64	-	-	33	33	-	64

TABLE 12

Summary of Inclusivity Hybridization Results of Shigella Specific Fragments and Capture/Detection Oligonucleotide Probes Included in Probe Sets 1 and 1 to Shigella Serotypes (++ SIGNAL USED AS CUT-OFF FOR DETECTABILITY).

Probe Set 1 includes oligonucleotides 1911/1500/1501, 1684/1685 and 1706/1707.
Probe Set 2 includes oligonucleotides 1706/1707 and 1864/437c.

SEROTYPE	TESTED	NT6	1911 1500 1501	NT19 -2	1684 1685	ompA 1706 1707	CR3 437c 1864	probe set I	probe set II
S. dysenteriae									
1	8	-	-	-	-	8	7	8	8
2	2	1	1	-	-	2	2	2	2
3	3	3	3	-	-	-	3	3	3
4	3	3	3	-	-	-	3	3	3
5	1	1	1	-	-	-	1	1	1
6	1	1	1	-	-	-	1	1	1
7	1	1	1	-	-	-	1	1	1
8	1	1	1	-	-	-	1	1	1
9	3	3	3	-	-	-	3	3	3
10	1	-	-	-	-	-	1	-	1
untyped	1	1	1	-	-	-	1	1	1
TOTAL	25							24	25

TABLE 12 : CONT'D

SEROTYPE	TESTED	NT6	1911 1500 1501	NT19 -2	1684 1685	ompA 1706 1707	CR3 437c 1864	probe set I	probe set II
S. flexneri									
1	5	-	-	5	5	-	5	5	5
2	2	-	-	2	2	-	2	2	2
3	5	2	2	5	5	-	5	5	5
4	4	-	-	4	4	-	4	4	4
5	2	1	1	2	2	-	2	2	2
6	3	3	3	-	-	-	3	3	3
untyped	36	16	16	36	36	-	36	36	36
TOTAL	57							57	57
S. boydii									
1	1	1	1	-	-	-	1	1	1
2	1	1	1	-	ND	ND	1	1	1
3	1	1	1	-	-	-	1	1	1
4	1	1	1	-	-	-	1	1	1
5	2	-	-	2	2	2	2	2	2
6	1	1	1	-	-	-	1	1	1
7	1	-	-	1	1	1	-	1	1

TABLE 12 : CONT'D

SEROTYPE	TESTED	NT6	1911 1500 1501	NT19 -2	1684 1685	ompA 1706 1707	CR3 437c 1864	probe set I	probe set II
S. boydii									
8	1	1	1	-	-	-	1	1	1
9	2	-	-	2	2	2	1	2	2
10	3	3	3	-	-	-	3	3	3
11	2	-	-	2	2	2	1	2	2
12	1	-	-	-	-	1	1	1	1
13	2	-	-	-	-	-	-	-	-
14	2	2	2	-	-	-	2	2	2
15	1	-	-	-	-	1	-	1	1
16	1	-	-	1	1	1	1	1	1
17	1	-	-	1	1	1	1	1	1
18	1	1	1	-	-	-	1	1	1
untyped	2	2	2	-	-	-	2	2	2
TOTAL	27							25	25
S. sonnei									
1	64	64	64	33	33	-	64	64	64

TABLE 13

SUMMARY OF HYBRIDIZATION RESULTS OF SHIGELLA PROBES, WHICH ARE INCLUDED IN PROBE SETS 1 AND 2, TO ENTEROINVASIVE E. COLI AND COMPETITOR ORGANISMS FOUND IN STOOL (+ SIGNAL USED AS CUT-OFF FOR DETECTABILITY).

	E. COLI (from Table 6)			OTHER ORGANISMS FOUND IN STOOL (from Tables 7,8)	
FRAGMENT (OLIGONUCLEOTIDES)	EIEC 5	EVEC 43	Non-EVEC 18	Cyto-dot 91	DNA-dot 91
PROBE SET 1					
NT6 (1500/1501/1911)	5	-	-	-	-
NT19-2 (1684/1685)	1	-	-	-	-
OMP A (1706/1707)	-	-	-	2	-
PROBE SET 2					
OMP A (1706/1707)	-	-	-	2	-
Class 3R (1864/437c)	3	-	-	-	-

Note: EIEC - Enteroinvasive E. coli.
EVEC - Enterovirulent E. coli includes isolates of Enterotoxigenic E. coli, Enteropathogenic E. coli O157 serotypes and non-O157 serotypes.
Non-EVEC - E. coli isolates not associated with disease.
The two competitor organisms which are weakly detected by the ompA probes are both Escherichia fergusonii.

Equivalents

Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed within the scope of this invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Parodos, Kyriaki
McCarty, Janice

(ii) TITLE OF INVENTION: Nucleic Acid Probes for the Detection of Shigella

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: U.S.A.
(F) ZIP: 60680

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US92/06617
(B) FILING DATE: 28-JUL-92

(vii) PRIOR APPLICATION DATA:
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(C) REFERENCE/DOCKET NUMBER: GTR90-04 PCT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATCCAACCG CAGTAATAAA CTGAATCCCT CGCATGGCTT GCAGCGCCTC TACTACCGGA 60

TACAGCCTCC ATTCGGTAAC NGCCTCCTTG AGGGCGGATT CCAGCCGTTC ACATTGTGCC 120

TGCCGATCTT CTATTGTACG ACGGTGTTCG TCAAAAGCTA ATTG 164

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 796 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CATCAGAAAT CTAAGCAGAA GTCTTTCCGT GTTTCTCAGA AATGGGGGCA ACGTGCAAAA 60

CTTGCCCTTG CTGGTGAACA ACGTCTTACA AAGATGGTTC CTGGATGGAT TGACCCTGAG 120

ACTTTTAAAC TCAATGAACA CGCTGAGACT GTGAGATTGA TATTCAAACT GCTGCTAGAT 180

GGTGAAAGTC TGCATAACAT TGCACGTCAC CTTCAAAGCA ACGGTATAAA GTCGTTTAGT 240

CGCCGTAAAG ATGCTAATGG GTTCTCTGTT CACTCTGTAC GCACATTCTA AGGTCAGAGC 300

AACAATAGGC ACGTTACCAG CATCACAACG TAATGACCGC CCCGCTATAC CGAACTACTA 360

CGAAGGTGTT GTAGATATAC CAACGTTCAA TAAAGCTCAA GAGATTCTCG ACAAGAATCG 420

TAAAGGCCGT ACACCTGCAA GTGACAACCC ACTAACGATT AACATCTTCA AAGGTCTGTT 480

TAGGTGTCAG TGTGGGGCTA GTGTCCATCC TACCGGAACA AAGAATAAGT ATGCTGGGGT 540

CTACAGGTGC AATAACCACT TAGACGGTCG CTGTGATGTT CCACCGTTGA AGCGTAAACC 600

GTTTGACCGA TGGATGATTG ATAATTTTCT GGGGATGATT GACGTGGGGA ATGATGGAGA 660

ATCAGAGAGA AAGATTGCAG CGTTACAGCA TGAGGTTGAA ATTGTCACAG CCAGAATCAA 720

GAAACGTACC GCCCTACTTC TTGAGATGGA TGATATTGAT GAACTAAAAA TTCAGCTTAA 780

GGAACTGAAC CAGAAG 796

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 587 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATCTTTCTT CGAAGAAGAG AGCGCACCAA TACCCGCGCC CACGAGAGAG CCCAGACCTG 60

CGCCGATAGC AGATTTGCCT GCTTCGCGTT CGCCGGTGTA AGGGTTAGTT GTGCAGCCAG 120

ATACCGCCAG AGCGCCACTC ACTACGGAGG CAATAAGATA AACACGTTTG TTCATTGTTA 180

ATCCTTCCTA ACCTTTTTAT TCTTTGCCAC GGGTTCCGTG GCGGGAGATT ATGCCGCGTG 240

AACATGAAGA TGAGGTGTAC TGGCAATAGC GGACACTACC ATTTGTTCTT TTTTTAAGCA 300

GCCATCTGAT GATATTTTTC CCTGAAGGCT GCCGGGGAGA TATTCCCCAG ACGAGAGTGA 360

CGACGCTGAC GATTCTAGAA AATCTCAATG TATTCCCGTA TTACTGAGAT GGCTTCATCC 420

CGGTTATTAA AACGATAGTG GCTCAGGCTC TCATTTTTCA GCGTTCCCCA CAAGCTTTCC 480

ATCGGAGCGT TGTCGTAACA GTTACCTTTA CGCGACATTG ATGTTTTCAG ACCAGACTGC 540

TCCTGTATGA CCCGGTAATC GTATGCGCAG TACTGTGAAC CTCGATC 587

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 786 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATCAGAGTG GTGGATTAGC CCGGCAGTGG GCGCTGGCTC CTGAGCGCCA TAAACAGGGC 60

TTTACCTGTC AGCTCTTTTG TCATGCGCTC TCCCATGGCG TACGACAATT TCGCACGTAT 120

AAACATCTTT GATGCCAGCG AGGTACAACC ATCCCTCCTG TGTGGCAACA TACGTCAGGT 180

CCGCCACCCA GACCTGATTT GGTGCTGTAG GAGCGAACGT CTGGTTCAGC AGATTTGGCG 240

CAACTGGCAG ATTGTGGTTC GAGTTCGTAG TCGCTCTGAA CTTGCGTTCT GCTTACAGCG 300

TAGCTTAGCT CCTTACGAAG ACGTGCCAGT CGGTCACGAC CAACGATGAT GCCATTCTCT 360

GCCAGCTCCG TCTGGGAGCC GCCGGGTTTC CATATGTTTC GCGAGTGCGG ATATGTGCCA 420

CCTTAATCTC CAGTTTTAGC CGCTCATCAC TTTGTTTTCT GTCTGAGGGT TCATGCTGTA 480

CCCAGTTGTA ATAACCGCTC CTGGATACAC CAAATACTGA CACATCGCTT CAATGGGAAA 540

TTGTTGTCGC CATTGTTCGA TTAACGCGTA TTTTTCAGCG ACTCCTGTGC AAAATACGCT 600

GTTGCTTTTT TTAATATATC TCGCTCAAGG CGAGCTTCAT TTAACGCCTT ACGCAGTCGC 660

AGAATTTCAG ATTCCAGTTC AGCCACCGTG CGGGAACCAG GAGTACCGAG CCCTTTTCTG 720

GCGGCGGTAA CCCATTGTCC TAAAGTGCCT TCAGGAAGAG ATAATCGGGA AGCGCCTTCA 780

CTGATC 786

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1174 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGTTGGACTA TAGGTGTACT GGCAATACGG ACACACCATT TGTTCTTTTT TTAAGCAGCA 60

TCTGATGATA TTTTTCCCTG AAGGCTGCCG GGGAGATATT CCCCAGACGA GAGTGACGAC 120

GCTGACGATT GTAGAAAATC TCAATGTATT CCCGTATTAC TGAGATGGCT TCATCCCGGT 180

TATTAAAACG ATAGTGGCTC AGGCTCTHAT TTTTCAGCGT TCCCCANAAG CTTTCCATCG 240

GAGCGTTGTC GTAACAGTTA CCTTTACGCG ACATTGATGT TTTCAGACCA AACTGCTCCT 300

GTATGACCCG GTAATCGTAT GCGCAGTACT GTGAACCTCG ATCAGAGTGG TGGATTAGCC 360

CGGCAGGNGG GCGCTGGCTC CTGAGCGCCA TAAACAGGGC TTTACCTGTC AGCTCTTTTG 420

TCATGCGCTC TCCCATGCGT AGCCGACAAT TTCGCACGTA TAACATCTTT GATGCCAGCG 480

AGGTACACCA TCCCTCCTGT GTGGCANCAT ACGTCAGGTC CGCCACCCAG ACCTGATTTG 540

GTGCTGTAGG AGTGAACGTC TGGTTCAGCA GATTTGGCGC AACTGGCAGA TTGTGGTTCG 600

GGTTCGTAGT CGCTCTGAAC TTGCGTTTCT GCTTACAGCG TASCTTAGCT CCTTACGAAG 660

ACGTGCCAGT CGGTCACGCC AACGATGATG CCATTCTCTG CCAGCTCCGT CTGGAGCCGC 720

CGGGTTCCAT ATGTTDCGCR AGTGCGGATA TGTGCCACCT TAATCTCCAG TTTDAGCCGC 780

TCATCACTTT GTTDTCTGTC TGAGGGTTCA TGCTGTACCC AGTTGTAATA ACCGCTCCTG 840

GATACACCAA ATACCTGACA CATCGCTTSA ATDDDAAATT GTTGTCGCCA TTGTTCGATT 900

AACGCGNNNN NNNCAGCGAC TCCTGTGCAA AATACGCTGT TGCTTTTTTT AATATATCTC 960

GCTCAAGGCG AGCTTCATTT AACGCTTTAC GCAGTTGCAG AATTTCAGAT TCCAGTTCAG 1020

CCACCGTGCG GGAACCAGGA GTACCGAGCC CTTTTCTGGC GGCGGTAACC CATTGTCCTA 1080

AAGTGCCTTC AGGAAGAGAT AATCGGGAAG CGCCTTCGCT GATCGAAAGT TGATTTTCAA 1140

GAACCGTTCT GACAGCTTCG GCTTTGAACT CTGT 1174

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 64 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGTGGCATCA ACAATGGTGC GACCACCGAG CGAGATGAGG TGTACTGGCA ATAGCGGACA 60

CAAC 64

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1196 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATTGTAGAAA ATCTCAATGT ATTCCCGTAT TACTGAGATG GCTTCATCCC GGTTATTAAA 60

ACGATAGTGG CTCAGGCTCT HATTTTTCAG CGTTCCCCAN AAGCTTTCCA TCGGAGCGTT 120

GTCGTAACAG TTACCTTTAC GCGACATTGA TGTTTTCAGA CCAAACTGCT CCTGTATGAC 180

CCGGTAATCG TATGCGCAGT ACTGTGAACC TCGATCAGAG TGGTGGATTA GCCCGGCAGG 240

NGGGCGCTGG CTCCTGAGCG CCATAAACAG GGCTTTACCT GTCAGCTCTT TTGTCATGCG 300

CTCTCCCATG CGTAGCCGAC AATTTCGCAC GTATAACATC TTTGATGCCA GCGAGGTACA 360

CCATCCCTCC TGTGTGGCAN CATACGTCAG GTCGGCCACC CAGACCTGAT TTGGTGCTGT 420

AGGAGTGAAC GTCTGGTTCA GCAGATTTGG CGCAACGGC AGATTGTGGT TCGGGTTCGT 480

AGTCGCTCTG AACTTGCGTT TCTGCTTACA GCGTASCTTA GCTCCTTACG AAGACGTGCC 540

AGTCGGTCAC GCCAACGATG ATGCCATTCT CTGCCAGCTC CGTCTGGAGC CGCCGGGTTC 600

CATATGTTDN GCRAGTGCGG ATATGTGCCA CCTTAATCTC CAGTTTDAGC CGCTCATCAC 660

TTTGTTDTCT GTCTGAGGGT TCATGCTGTA CCCAGTTGTA ATAACCGCTC CTGGATACAC 720

CAAATACCTG ACACATCGCT TNAATGGGAA ATTGTTGTCG CCATTGTTCG ATTAACGCGN 780

ATTTTTCAGC GACTCCTGTG CAAAATACGC TGTTGCTTTT TTTNATATAT CTCGCTCAAG 840

GCGAGCTTCA TTTAACGCCT TACGCAGTTG CAGAATTTCA GATTCCAGTT CAGCCACCGT 900

GCGGGAACCA GGAGTACCGA GCCCTTTTCT GGCGGCGGTA ACCCATTGTC CTAAAGTGCC 960

TTCAGGAAGA GNTAATCGGG AAGCGCCTTC GCTGATCGAA AGTTGATTTT CAAGAACCGT 1020

TCTGACAGCT TCGGCTTTGA ACTCTTTAGA GTAACGTTGG TTTTTTCTGC TCATTATTAG 1080

CTCCTTCTGA TGCCATTCTA TTTCAGGAAG GAGTGTCCGT TAAACTCAGG CTACCTCAAG 1140

ATAAAGTTAT TAATTTCGAA GATCACATCT TCAATAGGTT TGCGGTCCAT ATTATC 1196

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 64 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AAAGCACAGA TTTTATAGCT AACTCGATGC TGGTGTGAGG TGTACTGGCA ATAGCGGACA 60

CTAC 64

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1188 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATTGTAGAAA ATCTCAATGT ATTCCCGTAT TACTGAGATG GCTTCATCCC GGTTATTAAA 60

ACGATAGTGG CTCAGGCTCT HATTTTTCAG CGTTCCCCAN AAGCTTTCCA TCGGAGCGTT 120

GTCGTAACAG TTACCTTTAC GCGACATTRA TGTTTTCAGA CCAGACTRCT CCTGTATGAC 180

CCGGTAATCG TATGCGCAGT ACTGTGAACC TCGATCAGAG TGGTGGATTA GCCCGGCAGG 240

NGGGCGCTGG CTCCTGAGCG CCATAAACAG GGCAAATCCT GTCAGCTCTW TTGTCATGCG 300

CTCTCCCATG CGTAGCCGAC TAATTTCGCA CGTATAACAT CTTTGATGCC AGCGAGGTAC 360

ACCATCCCTC CTGTGTGGCA NCATACGTCA GGTCCGCCAC CCAGACCTGA TTTGGTGCTG 420

TAGGAGCGAA CGTCTGGTTC AGCAGATTTG GCGCAACTGG CAGATTGTGG TTCGAGTTCG 480

TAGTCGCTCT GAACTTGCGT TTCTGCTTAC AGTGTAGCCT TAGCTCCTTA CGAAGACGTG 540

CCAGTCGGTC ACGCCAACGA TGATGCCATT CTCTGCCAGC TCCGTCTGGA GCCGCCGGGT 600

TCCATATGTT NCGCRAGTGC GGATATGTGC CACCTTAATC TCCAGTTTDA GCCGCTCATC 660

ACTTTGTTDT CTGTCTGAGG GTTCATGCTG TACCCAGTTG TAATAACCGC TCCTGGATAC 720

ACCAAATACC TGACACATCG CTTSAATGGG AAATTGTTRT CGCCATTGTT CGATTAACGC 780

GACTCCTCTG CAAAATACGC TGTTGCTTTT TTNNATATAT CTCGCTCAAG GCGAGCTTCA 840

TTTAACGCCT TACGCAGTTG CAGAATTTCA GATTCCAGTT CAGCCACCGT GCGGGAACCA 900

GGAGTACCGA GCCCTTTTCT GGCGGCGGTA ACCCATTGTC CTAAAGTGCC TTCAGGAAGA 960

GATAATCGGG AAGCGCCTTC ACTGATCGAA AGTTGATTTT CAGGAACCGT TCTGACAGCT 1020

TCGGCTTTGA ACTCTTKAGA GTAACGTTGG GTTTTTCTGC TCATTATTAG CTCCTTCTGA 1080

TGCCATTCTA TTTCAGGAAG GAGTGTCCGT TAAACTCAGG CTACCTCAGT GTGATCGGCG 1140

ATAAGCCCAG AACTCCGCTC CCAGACCTCC CTGCCAAAAG CAAAACCG 1188

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 630 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATCTCCTTAT GTTATGGAGA TTATTAAAAA GAATAACATT AGCGCTCTCG AACTGCATCG 60

TGCAATTGTT GAGTTGAGTA AAAATATGAA GTCGATTGAT GATAATGCCA GTAAGAAAAA 120

CGACAAGTCA TCATTGTATG TATCATGGAC TCTGAGTTTT ACTGCTCCAA CAAGTAAAGA 180

AGCTCACGAT GTGTTGTCTG GGTATATTAA TTATGTTTCT TCCCTTGTTG TAAGGGATTT 240

GATGGAAGAT ATAAGAAATA AACTAGAAGT TAAAACTAAT GTTGAAAAAG AAATTCTTGC 300

ACTGGATGAG ATAAAAATTA GAAACCAGCT GAATGCAGAT ATTCGACNCC TCAATTATTC 360

ACTGGAGGTT GCTAATGCGG CTGGAATAAA AAAACCTGTA TACAGCAATG GTCAGATTAT 420

GAAGGATGAC CCAGATTTTC CTGTGGCTCT CGGTTCTGAT GGTATAGCAA CTAAATTGAA 480

CATCAAAAAA TCAATCAAGG ATGTTTCGGA ATTGAGTGGG GAGTTGCGAA ATCGTCAATA 540

TGTTGTGAAT CAATTGGTTG TGGCGAAAGN GGGGGANGNN GANNNNANGC MANNNCAGNA 600

NCAANNGTGC CCAACGNNAC CGGNCAGAAA 630

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 388 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCACCACATT GATTGTCTGC CTGAAATAAC ACGAAAGGCA CTGCGTGAAC GCTATGTGGA 60

ACAGCTGGTG GCTACAGAGA ACAATGTTTC TGAAGTGAAA GCTGTTACCA GAAAAACACG 120

CAATCCTGAC GCTGTCCAGG CAATCGAAGC ATATCGCGGT TCTCCACAAC TGATGGAAGA 180

ACGCCTGAAT GCGCTGACCG AAAACCAGCG CTGGGTATCT GAAGCAAGAG CTGCGCTGGT 240

GGTGGAAGTG CTGAAGCTGG AAAGCGCCGG TAACCCCGGG CGACTGAAAG CCATTAACTT 300

TCTTGTTGAA AAAGCCCCTA AAGGTGAGCT GCCGGAGCGC CTGCAACAGG CCGCAGTTAA 360

CGCCAATGCA AAACGTGGCG CTAATCGT 388

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 184 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGACGACCT GGACGTGTAC ACTCGTCTGG GTGGTATGGT TTGGCGTGCA GACACCAAAG 60

CTCACAACAA TGTGACAGGT GAATCTGAGA AAAACCACGA TACCGGCGTT TCTCCGGTAT 120

TCGCAGGTGG TGTTGAATGG GCCATCACTC CTGAAATCGC TACCCGTCTG GAATACCAGT 180

GGAC 184

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 169 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTGACGACCT GGACATCTAC ACTCGTCTGG GTGGCATGGT ATGGCGTGCA GACACTAAAT 60

CCAACGTTTA TGGTAAAAAC CACGACACCG GCGTTTCTCC GGTCTTCGCT GGCGGTGTTG 120

AGTACGCGAT CACTCCTGAA ATCGCTACCC GTCTGGAATA CCAGTGGAC 169

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTGCAGCGCC TCTACTACCG GATACAGCCT CCATT 35

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCTCCTTCAG GGCGGATTCC AGCCGTTCAC ATTGT 35

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCGATCTTCT ATTGTACGAC GGTGTTCGTC AAAAGCTAAT 40

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGGTGAACA ACGTCTTACA AAGATGGTTC CTGGATGGAT T 41

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGTCTTTCCG TGTTTCTCAG AAATGGGGGC AACGTGCAAA A 41

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CCACCGTTGA AGCGTAAACC GTTTGACCGA TGGAT 35

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCTGGGGTCT ACAGGTGCAA TAACCACTTA GACGGT 36

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGATGATGCC ATTCTCTGCC AGCTCCGTCT GGGAGCCGCC GGGTTTCC 48

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGAGCAGTCT GGTCTGA 17

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCTGTGGCTC TCGGTTCTGA TGGTATAGCA ACTAAAT 37

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CAAGGATGTT TCGGAATTGA GTGGGGAGTT GCGAAAT 37

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CAGGCAATCG AAGCATATCG CGGTTCTCCA CAACT 35

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TGAATGCGCT GACCGAAAAC CAGCGCTGGG TATCT 35

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTGATGGCCC ATTCAACACC ACCTGCGAAT ACCGG 35

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTCAGATTCA CCTGTCACAT TGTTGTGAGC TTTGG 35

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AATCCATCCA GGAACCATCT TTGTAAGACG TTGTTCACCA G 41

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTTTGCACGT TGCCCCCATT TCTGAGAAAC ACGGAAAGAC T 41

CLAIMS

1. An exclusively chromosomal Shigella specific fragment identified by subtractive hybridization using S. flexneri as "target" DNA and a complex DNA competitor mix as competitor DNA.

2. A Shigella specific fragment identified by subtractive hybridization using S. sonnei as "target" DNA and a mixture of non-pathogenic E. coli YMC DNA and pBR322 vector DNA as competitor DNA.

3. A Shigella specific fragment derived from a chromosomal sequence of Shigella sonnei, the Shigella specific fragment selected from the group consisting of NT-6 (nucleotides 1-124 of SEQ ID NO:1), NT11-2 (SEQ ID NO:2), NT14 (SEQ ID NO:4) and NT15 (SEQ ID NO:3).

4. A Shigella specific fragment derived from a chromosomal sequence of Shigella flexneri, the Shigella specific fragment selected from the group consisting of NT18-1a (SEQ ID NO:10) and NT19-2 (SEQ ID NO:11).

5. A probe for the detection of a member of the genus Shigella wherein the probe is capable of detecting at least one untyped isolate or at least one isolate of a serotype of each of the

four species of the genus Shigella selected from the group consisting of:

a) (SEQ ID NO:1);

b) probes derived from SEQ ID NO:1;

c) NT11-2 (SEQ ID NO:2);

d) probes derived from NT11-2; and

e) homologues of (a) through (d).

6. A probe of Claim 5 for the detection of a member of the genus Shigella wherein the probe is capable of detecting at least one untyped isolate or at least one isolate of a serotype of each of the four species of the genus Shigella, wherein the probe is selected from the group consisting of:

a) 1500 (SEQ ID NO:14),
5'-TTGCAGCGCCTCTACTACCGGATACAGCCTCCATT-3';

b) 1501 (SEQ ID NO:15),
5'-CCTCCTTCAGGGCGGATTCCAGCCGTTCACATTGT-3';

c) 1911 (SEQ ID NO:16),
5'-CCGATCTTCTATTGTACGACGGTGTTCGTCAAA-AGCTAAT-3';

d) 1682 (SEQ ID NO:17),
5'-CTGGTGAACAACGTCTTACAAAGATGGTTCCTG-GATGGATT-3';

e) 1683 (SEQ ID NO:18),
5'-AGTCTTTCCGTGTTTCTCAGAAATGGGGGCAAC-GTGCAAAA-3';

f) 1708 (SEQ ID NO:19),
5'-CCACCGTTGAAGCGTAAACCGTTTGACCGATGG-AT-3'; and

g) complements of (a) through (f).

7. A probe capable of detecting at least one isolate of each of serotypes 1-5 of Shigella flexneri selected from the group consisting of:

a) NT18-1a (SEQ ID NO:10);

b) probes derived from NT18-1a;

c) homologues of (a) and (b);

d) NT19-2 (SEQ ID NO; 11);

e) probes derived from NT19-2; and

f) homologues of (d) and (e).

8. A probe of Claim 7 capable of detecting of at least one isolate of each of serotypes 1-5 of Shigella flexneri wherein the probe is selected from the group consisting of:

a) 1712 (SEQ ID NO:23),
5'-CCTGTGGCTCTCGGTTCTGATGGTATAGCAACT-AAAT-3';

b) 1713 (SEQ ID NO: 24),
5'-CAAGGATGTTTCGGAATTGAGTGGGGAGTTGCG-AAAT-3'; and

c) complements of (a) and (b).

9. A probe of Claim 7 (d) through (f) which further detects a member of the genus Shigella selected from the group consisting of S. boydii serotype 5, S. boydii serotype 7, S. boydii serotype 9, S. boydii serotype 11, S. boydii serotype 16, S. boydii serotype 17, and S. sonnei serotype 1.

10. A probe of Claim 9 selected from the group consisting of:

a) 1684 (SEQ ID NO:25),
5'-CAGGCAATCGAAGCATATCGCGGTTCTCCACAACT-3';

b) 1685 (SEQ ID NO: 26),
5'-TGAATGCGCTGACCGAAAACCAGCGCTGGGTATCT-3';
and

c) complements of (a) and (b).

11. An oligonucleotide probe for the detection of at least one isolate of Shigella selected from the group consisting of S. dysenteriae serotypes 1 and 2, and S. boydii serotypes 5, 7, 9, 11, 12, 15, 16, and 17, the oligonucleotide probe comprising a sequence selected from the group consisting of:

a) 1707 (SEQ ID NO:28),
5'-CTCAGATTCACCTGTCACATTGTTGTGAGCTTTGG-3';

b) 1706 (SEQ ID NO:27);
5'-GTGATGGCCCATTCAACACCACCTGCGAATACCGG-3';

c) complements of (a) and (b); and

d) homologues of (a) through (c).

12. A substantially inclusive oligonucleotide probe for the detection of a member of the genus Shigella comprising a sequence selected from the group consisting of:

a) 437 (SEQ ID NO:21),
5-'CGATGATGCCATTCTCTGCCAGCTCCGTCTGG-
GAGCCGCCGGGTTTCC-3';

b) 1864 (SEQ ID NO:22),
5'-GGAGCAGTCTGGTCTGA-3';

c) complements of (a) and (b); and
d) homologues of (a) and (b).

13. A substantially inclusive capture/detector probe pair for the detection of a member of the genus Shigella, comprising a substantially inclusive capture probe and a detector probe wherein the capture probe is selected from the group consisting of:
a) 1864 (SEQ ID NO:22); and
b) complement of 1864;
the detector probe having an oligonucleotide sequence derived from the same strand of the S. sonnei sequence comprising fragments NT14 (SEQ ID NO:4) and NT15 (SEQ ID NO:3) as the capture probe.

14. A substantially inclusive capture/detector probe pair of Claim 13, the probe pair also being exclusive of exclusivity organisms commonly found in stool and substantially exclusive of non-EIEC Enterobacteriaceae.

15. A capture/detector probe pair of Claim 13 wherein the capture probe is 1864 (SEQ ID NO:22) and the detector probe is the complement of 437.

16. A capture/detector probe pair of Claim 13 wherein the capture probe is the complement of 1864 and the detector probe is probe 437 (SEQ ID NO:21).

17. A substantially inclusive probe set for the detection of a member of the genus Shigella, which further detects EIEC, comprising at least three capture/detector probe pairs, the first pair of probes selected from the group consisting of oligonucleotides having sequences derived from a selected strand of SEQ ID NO:1 and homologues thereof, the second pair of probes selected from the group consisting of oligonucleotides having sequences derived from a selected strand of fragment NT19-2 (SEQ ID NO:11) and homologues thereof, and the third pair of probes selected from the group consisting of oligonucleotides having sequences derived from a selected strand of the S. dysenteriae ompA sequence which spans nucleotides from position 893 through 1076 (SEQ ID NO:12) and homologues thereof.

18. A substantially inclusive probe set of Claim 17, comprising at least three capture/detector probe pairs, the first pair of probes having oligonucleotide sequences derived from a selected strand of SEQ ID NO:1, the second pair of probes having oligonucleotide sequences derived from a selected strand of fragment NT19-2 (SEQ ID NO: 11), and the third pair of probes having oligonucleotide sequences derived

from a selected strand of the S. dysenteriae ompA sequence which spans nucleotides from position 893 through 1076 (SEQ ID NO:12).

19. A substantially inclusive probe set of Claim 18, the probe set also being exclusive of exclusivity organisms commonly found in stool and substantially exclusive of non-EIEC Enterobacteriaceae.

20. A substantially inclusive probe set of Claim 19 wherein the first pair of probes is selected from the group consisting of probes 1911 (SEQ ID NO:16), 1500 (SEQ ID NO:14) and 1501 (SEQ ID NO:15), the second pair of probes consists of probes 1684 (SEQ ID NO:25) and 1685 (SEQ ID NO:26), and the third pair of probes consists of probes 1706 (SEQ ID NOL:27) and 1707 (SEQ ID NO:28).

21. A substantially inclusive probe set for the detection of a member of the genus Shigella, which further detects EIEC, comprising at least three capture/detector probe pairs, the first pair selected from the group consisting of:

a) 1911 (SEQ ID NO:16);

b) 1500 (SEQ ID NO:14); and

c) 1501 (SEQ ID NO:15);

the second pair selected from the group consisting of:

a) 1684 (SEQ ID NO:25); and

b) 1685 (SEQ ID NO:26);
the third pair selected from the group consisting of:
a) 1706 (SEQ ID NO:27); and
b) 1707 (SEQ ID NO:28).

22. A substantially inclusive probe set for the detection of a member of the genus Shigella, which further detects EIEC, comprising at least two capture/detector probe pairs, the first pair of probes selected from the group consisting of oligonucleotides having sequences derived from a selected strand of the sequence comprising S. sonnei fragments NT14 (SEQ ID NO:4) and NT15 (SEQ ID NO:3) and homologues thereof, and the second pair of probes selected from the group consisting of oligonucleotides having sequences derived from a selected strand of the S. dysenteriae ompA sequence which spans nucleotides from position 893 through 1076 (SEQ ID NO:12) and homologues thereof.

23. A substantially inclusive probe set of Claim 22, comprising at least two capture/detector probe pairs, the first pair of probes having oligonucleotide sequences derived from a selected strand of the sequence comprising S. sonnei fragments NT14 (SEQ ID NO:4) and NT15 (SEQ ID NO:3), and the second pair of probes having oligonucleotide sequences derived from a selected strand of the S. dysenteriae ompA sequence which spans nucleotides from position 893 through 1076 (SEQ ID NO:12).

24. A substantially inclusive probe set of Claim 23, the probe set also being exclusive of exclusivity organisms commonly found in stool and substantially exclusive of non-EIEC Enterobacteriaceae.

25. A substantially inclusive probe set of Claim 24 wherein the first pair of probes consists of probes 1864 (SEQ ID NO:22) and the complement of 437, and the second pair of probes consists of probes 1706 (SEQ ID NO:27) and 1707 (SEQ ID NO:28).

26. A substantially inclusive probe set for the detection of a member of the genus Shigella, which further detects EIEC, comprising at least two capture/detector probe pairs, wherein the first pair is 1864 (SEQ ID NO:22) and the complement of 437 and the second pair is 1706 (SEQ ID NO:27) and 1707 (SEQ ID NO:28).

27. An oligonucleotide probe derived from Shigella specific fragment NT-6 (nucleotides 1-124 of SEQ ID NO:1)which substantially retains the inclusivity behavior of NT-6.

28. An oligonucleotide probe of Claim 27 which has improved exclusivity behavior for non-EIEC Enterobacteriaceae and is exclusive of the exclusivity organisms commonly found in stool.

29. An oligonucleotide probe of Claim 28 comprising a sequence selected from the group consisting of:

a) 1911 (SEQ ID NO:16),
5'-CCGATCTTCTATTGTACGACGGTGTTCGTCAAA-AGCTAAT-3';

b) 1500 (SEQ ID NO:14),
5'-TTGCAGCGCCTCTACTACCGGATACAGCCTCCATT-3';

c) 1501 (SEQ ID NO:15),
5'-CCTCCTTCAGGGCGGATTCCAGCCGTTCACATTGT-3';

d) complements of (a) through (c); and

e) homologues of (a) through (d).

30. An oligonucleotide probe derived from Shigella specific fragment NT11-2 (SEQ ID NO:2) which substantially retains the inclusivity behavior of NT11-2.

31. An oligonucleotide probe of Claim 30 which substantially retains the exclusivity behavior of NT11-2 (SEQ ID NO:2) towards non-EIEC Enterobacteriaceae.

32. An oligonucleotide probe of Claim 31 comprising a sequence selected from the group consisting of:

a) 1682 (SEQ ID NO:17),

5′-CTGGTGAACAACGTCTTACAAAGATGGTTCCTG-GATGGATT-3′;

b) complement of 1682 (SEQ ID NO:29),

5′-AATCCATCCAGGAACCATCTTTGTAAGACGTTG-TTCACCAG-3′; and

c) homologues of (a) and (b).

33. An oligonucleotide probe derived from Shigella specific fragment NT11-2 (SEQ ID NO:2) which moderately retains the inclusivity behavior of NT11-2.

34. An oligonucleotide probe of Claim 33 which substantially retains the exclusivity behavior of NT11-2 (SEQ ID NO:2) towards non-EIEC Enterobacteriaceae.

35. An oligonucleotide probe of Claim 34 comprising a sequence selected from the group consisting of:

a) 1683 (SEQ ID NO:18),

5′-AGTCTTTCCGTGTTTCTCAGAAATGGGGGCAAC-GTGCAAAA-3′;

b) complement of 1683 (SEQ ID NO:30),

5′-TTTTGCACGTTGCCCCCATTTCTGAGAAACACGG-AAAGACT-3′; and

c) homologues of (a) and (b).

36. An oligonucleotide probe derived from Shigella specific fragment NT11-2 (SEQ ID NO:2) which partially retains the inclusivity behavior of NT11-2.

37. An oligonucleotide probe of Claim 36 which substantially retains the exclusivity behavior of NT11-2 (SEQ ID NO:2) towards non-EIEC Enterobacteriaceae.

38. An oligonucleotide probe of Claim 37 comprising a sequence selected from the group consisting of:

a) 1708 (SEQ ID NO:19), 5'-CCACCGTTGAAGCGTAAACCGTTTGACCGATGG-AT-3'; and

b) 1709 (SEQ ID NO:20), 5'-GCTGGGGTCTACAGGTGCAATAACCACTTAGAC-GGT'-3';

c) complements of (a) and (b); and

d) homologues of (a) through (c).

39. An oligonucleotide probe derived from Shigella specific fragment NT18-1a (SEQ ID NO:10) which substantially retains the inclusivity behavior of NT18-1a.

40. An oligonucleotide probe of Claim 39 which substantially retains the exclusivity behavior of NT18-1a (SEQ ID NO:10) towards non-EIEC Enterobacteriaceae.

41. An oligonucleotide probe of Claim 40 comprising a sequence selected from the group consisting of:

a) 1712 (SEQ ID NO:23),
5'-CCTGTGGCTCTCGGTTCTGATGGTATAGCAACT-AAAT-3';

b) 1713 (SEQ ID NO:24),
5'-CAAGGATGTTTCGGAATTGAGTGGGGAGTTGCG-AAAT-3';

c) complements of (a) and (b); and

d) homologues of (a) through (c).

42. An oligonucleotide probe derived from Shigella specific fragment NT19-2 (SEQ ID NO:11) which substantially retains the inclusivity behavior of NT19-2.

43. An oligonucleotide probe of Claim 42 which substantially retains the exclusivity behavior of NT19-2 (SEQ ID NO:11) towards non-EIEC Enterobacteriaceae.

44. An oligonucleotide probe of Claim 43 comprising a sequence selected from the group consisting of:

a) 1684 (SEQ ID NO:25),
5'-CAGGCAATCGAAGCATATCGCGGTTCTCCACAACT-3';

b) 1685 (SEQ ID NO:26),
5'-TGAATGCGCTGACCGAAAACCAGCGCTGGGTATCT-3';

c) complements of (a) and (b); and

d) homologues of (a) through (c).

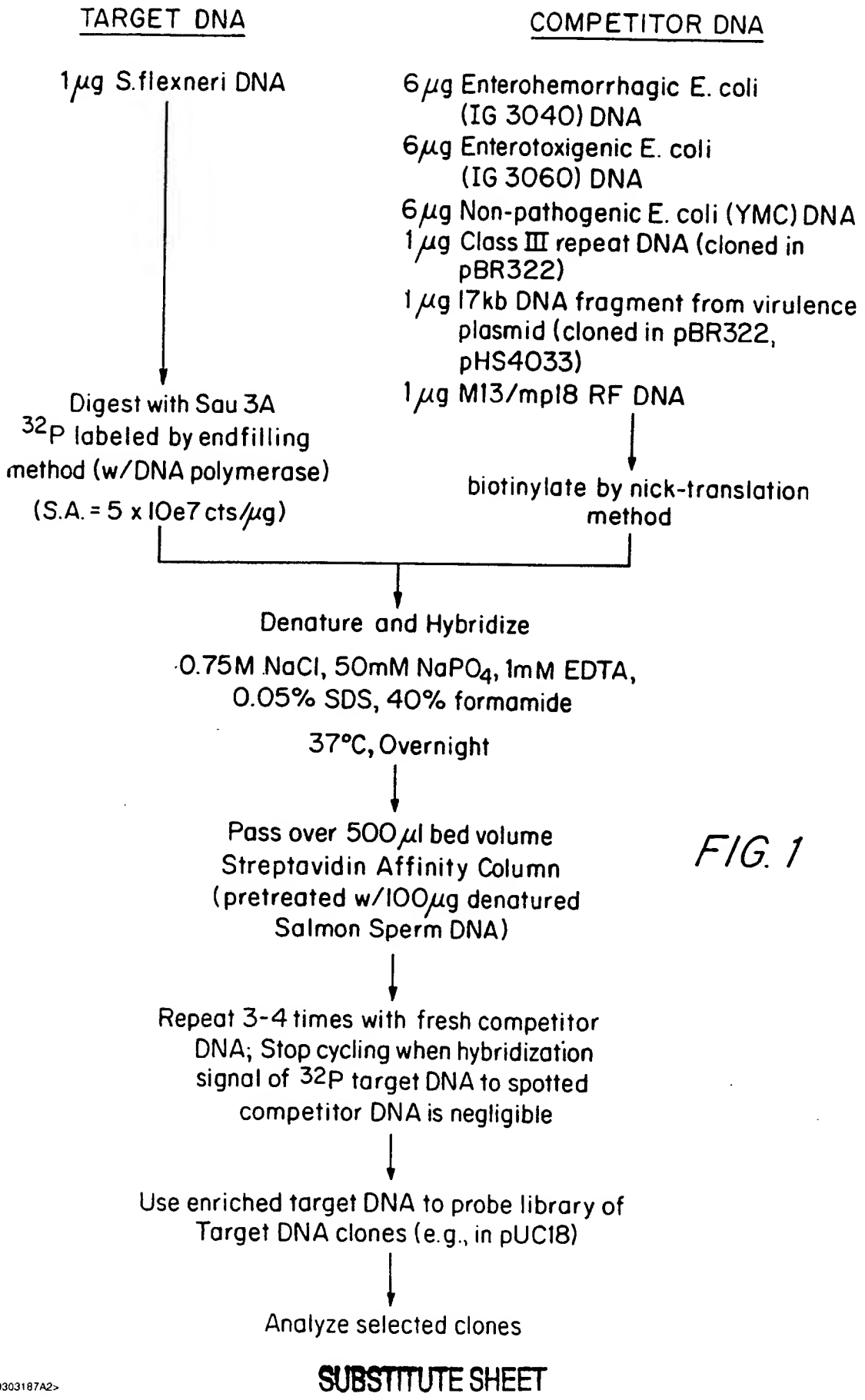
TARGET DNA
1μg S.flexneri DNA
Digest with Sau 3A
32P labeled by endfilling method (w/DNA polymerase)
(S.A. = 5 x 10e7 cts/μg)
COMPETITOR DNA
6μg Enterohemorrhagic E. coli (IG 3040) DNA
6μg Enterotoxigenic E. coli (IG 3060) DNA
6μg Non-pathogenic E. coli (YMC) DNA
1μg Class III repeat DNA (cloned in pBR322)
1μg 17kb DNA fragment from virulence plasmid (cloned in pBR322, pHS4033)
1μg M13/mp18 RF DNA
biotinylate by nick-translation method
Denature and Hybridize
0.75M NaCl, 50mM NaPO4, 1mM EDTA, 0.05% SDS, 40% formamide
37°C, Overnight
Pass over 500μl bed volume Streptavidin Affinity Column (pretreated w/100μg denatured Salmon Sperm DNA)
Repeat 3-4 times with fresh competitor DNA; Stop cycling when hybridization signal of 32P target DNA to spotted competitor DNA is negligible
Use enriched target DNA to probe library of Target DNA clones (e.g., in pUC18)
Analyze selected clones

FIG. 1

FIGURE 2

```
                5'
NT6             aaTCCAACCGCAGTAATAAACTGAATCCCTCGCATGGCTTGCAGCGCCTCTACTA
Probe 1500                                      TTGCAGCGCCTCTACTA

NT6 cont'd      CCGGATACAGCCTCCATTCGGTAACNgCCTCCTTCAGGGCGGATTCCAGCCGTTC
1500 cont'd     CCGGATACAGCCTCCATT
Probe 1501                      CCTCCTTCAGGGCGGATTCCAGCCGTTC

                          Sau3A
                          | | |
NT6 cont'd      ACATTGTGCCTGCCGATCTTCTATTGTACGACGGTGTTCGTCAAAAGCTAATTG  3'
1501 cont'd     ACATTGT
Probe 1911                CCGATCTTCTATTGTACGACGGTGTTCGTCAAAAGCTAAT
```

FIGURE 3A

```
                5'
NT11-2           CATCAGAAATCTAAGCAGAAGTCTTTCCGTGTTTCTCAGAAATGGGGGCAACGTGC
Probe 1683                                AGTCTTTCCGTGTTTCTCAGAAATGGGGGCAACGTGC

NT11-2 cont'd    AAAACTTGCCCTTGCTGGTGAACAACGTCTTACAAAGATGGTTCCTGGATGGATTG
1683 cont'd      AAAA
Probe 1682                          CTGGTGAACAACGTCTTACAAAGATGGTTCCTGGATGGATT

NT11-2 cont'd    ACCCTGAGACTTTTAAACTCAATGAACACGCTGAGACTGTGAGATTGATATTCAAA

NT11-2 cont'd    CTGCTGCTAGATGGTGAAAGTCTGCATAACATTGCACGTCACCTTCAAAGCAACGG

NT11-2 cont'd    TATAAAGTCGTTTAGTCGCCGTAAAGATGCTAATGGGTtCTCTGTTCACTCTGTAC

NT11-2 cont'd    GCACATTCTAAGGTCAGAGCAACAATAGGCACGTTACCAGCATCACAACGTAATGA

NT11-2 cont'd    CCGCCCCGCTATACCGAACTACTACGAAGGTGTTGTAGATATACCAACGTTCAATA

NT11-2 cont'd    AAGCTCAAGAGATTCTCGACAAGAATCGTAAAGGCCGTACACCTGCAAGTGACAAC
```

FIGURE 3B

```
NT11-2 cont'd  CCACTAACGATTAACATCTTCAAAGGtCTGTTTAGGTGTCAGTGTGGGGCTAGTGT

NT11-2 cont'd  CCATCCTACCGGAACAAAGAATAAGTATGCTGGGGTCTACAGGTGCAATAACCACT
Probe 1709                                    GCTGGGGTCTACAGGTGCAATAACCACT

NT11-2 cont'd  TAGACGGTCGCTGTGATGTTCCACCGTTGAAGCGTAAACCGTTTGACCGATGGATG
1709 cont'd    TAGACGGT
Probe 1708                               CCACCGTTGAAGCGTAAACCGTTTGACCGATGGAT

NT11-2 cont'd   ATTGATAATTTTCTGGGGATGATTGACGTGGGGAATGATGGAGAATCAGAGAGAA

NT11-2 cont'd   AGATTGCAGCGTTACAGCATGAGGTTGAAATTGTCACAGCCAGAATCAAGAAACG

NT11-2 cont'd   TACCGCCCTACTTCTTGAGATGGATGATATTGATGAACTAAAAATTCAGCTTAAG

                                3'
NT11-2 cont'd   GAACTGAACCAGAAG
```

FIGURE 4A

```
                5'
NT15            GATCTTTCTTCGAAGAAGAGAGCGCACCAATACCCGCGCCCACGAGAGAGCCCAG

NT15            ACCTGCGCCGATAGCAGATTTGCCTGCTTCGCGTTCGCCGGTGTAAGGGTTAGTT

NT15            GTGCAGCCAGATACCGCCAGAGCGCCACTCACTACGGAGGCAATAAGATAAACAC

NT15            GTTTGTTCATTGTTAATCCTTCCTAACCTTTTTATTCTTTGCCACGGGTTCCGTG
E.c. 2                                                              TGTGG
S.f                                                                 AAAGC

NT15            GCGGGAGATTATGCCGCGTGAACATGAAGATGAGGTGTACTGGCAATAGCGGACA
E.c. 1                                    AGTTGGACTATAgGTgTACTgGCAATA-CgGACA
E.c. 2          CATCAACAATGGTGCGACCACCGAGCGAGATGAGGTGTACTGGCAATAGCGGACA
S.f.            ACAGATTTTATAGCTAACTCGATGCTGGTGTGAGGTGTACTGGCAATAGCGGACA
                                          ***
                                    Left end of repeat.

NT15            CTACCATTTGTTCTTTTTTTAAGCAGCCATCTGATGATATTTTTCCCTGAAGGCT
E.c. 1          C-AcCAtTTGTTctttttTTTAAGCAG-CATCTGATGATAttTTTCCCTGAAGGCT
E.c. 2          CAAC----------------------------------------------------
S.f.            CTAC----------------------------------------------------
```

FIGURE 4B

```
NT15        GCCGGGGAGATATTCCCCAGACGAGAGTGACGACGCTGACGATTCTAGAAAATCTC
E.c. 1      GcCggGGAGATATTcCCCAGACGAGAGTGACGACGCTGACGATTGTAGAAAATCTC
E.c. 2      ----------------------------------------ATTGTAGAAAATCTC
S.f.        ----------------------------------------ATTGTAGAAAATCTC

NT15        AATGTATTCCCGTATTACTGAGATGGCTTCATCCCGGTTATTAAAACGATAGTGGC
E.c. 1      AATGTATtCCCGTATTACTGAGATGGCTTCATCCCGGTTATTAAAACGATAGTGGC
E.c. 2      AATGTATtCCCGTATTACTGAGATGGCTTCATCCCGGTTATTAAAACGATAGTGGC
S.f.        AATGTATtCCCGtATTACTGAGATGGCTTCATCCCGGTTATTAAAACGATAGTGGC

NT15        TCAGGCTCTCATTTTTCAGCGTTCCCCACAAGCTTTCCATCGGAGCGTTGTCGTAA
E.c. 1      TCAGGCTCtHATTTTTCAGCGTTCCCCANAAGCTTTCCATCGGAGCGTTGTCGTAA
E.c. 2      TCAGGCTCtHATTTTTCAGCGTTCCCCANAAGCTTTCCATCGGAGCGTTGTCGTAA
S.f.        TCAGGCTCtHATTTTTCAGCGTTCCCCANAAGCTTTCCATCGGAGCGTTGTCGTAA

NT15        CAGTTACCTTTACGCGACATTGATGTTTTCAGACCAGACTGCTCCTGTATGACCCGG
E.c. 1      CAGTTACCTTTACGCGACATTGATGTTTTCAGACCAAACTGCTCCTGTATGACCCGG
E.c. 2      CAGTTACCTTTACGCGACATTGATGTTTTCAGACCAAACTGCTCCTGTATGACCCGG
S.f.        CAGTTACCTTTACGCGACATTRATGTTTTCAGACCAGACTRCTCCTGTATGACCCGG
Probe 1864                                  3' AGTCTGGTCTGACGAGG 5'
```

FIGURE 4C

```
NT15          TAATCGTATGCGCAGTACTGTGAACCTCGATC
NT14                                        GATCAGAGTGGTGGATTAGCCCGGCAG-
E.c. 1        TAATCGTATGCGCAGTACTGTGAACCTCGATCAGAGTGGTGGATTAGCCCGGCAGG
E.c. 2        TAATCGTATGCGCAGTACTGTGAACCTCGATCAGAGTGGTGGATTAGCCCGGCAGG
S.f.          TAATCGTATGCGCAGTACTGTGAACCTCGATCAGAGTGGTGGATTAGCCCGGCAGG

NT14          TGGGCGCTGGCTCCTGAGCGCCATAAACAGGGCTTTACCTGTCAGCTCTTTTGTCA
E.c. 1        NGGGCGCTGGCTCCTGAGCGCCATAAACAGGGCTTTACCTGTCAGCTCTTTTGTCA
E.c. 2        NGGGCGCTGGCTCCTGAGCGCCATAAACAGGGCTTTACCTGTCAGCTCTTTTGTCA
S.f.          NGGGCGCTGGCTCCTGAGCGCCATAAACAGGGCAAATCCTGTCAGCTCTWTTGTCA

NT14          TGCGCTCTCCCATGGCGTA--CGAC-AATTTCGCACGTATAAACATCTTTGATGCC
E.c. 1        TGcGCTCTCCCATG-cGTAGCCGAC-AATTTCGCACGTATAA-CATCTTTGATGCC
E.c. 2        TGCGCTCTCCCATG-cGTAGCCGAC-AATTTCGCACGTATAA-CATCTTTGATGCC
S.f.          TGCGCTCTCCCATG-cGTAGCCGACtAATTTCGCACGTATAA-CATCTTTGATGCC

NT14          AGCGAGGTACAACCATCCCTCCTGTGTGGCAACATACGTCAGGTCCGCCACCCAGA
E.c. 1        AGCGAGGTACA-CCATCCCTCCTGTGTGGCAnCATACGTCAGGTCCGCCACCCAGA
E.c. 2        AGCGAGGTACA-CCATCCCTCCTGTGTGGCAnCATACGTCAGGTCCGCCACCCAGA
S.f.          AGCGAGGTACA-CCATCCCTCCTGTGTGGCAnCATACGTCAGGTCCGCCACCCAGA
```

FIGURE 4D

```
NT14          CCTGATTTGGTGCTGTAGGAGCGAACGTCTGGTTCAGCAGATTTGGCGCAACTGGC
E.c. 1        CCTGaTTTGGTGCTGTAGGAgTGAACGTCTGGTTCAGCAGATTTGGCGCAACTGGC
E.c. 2        CCTGaTTTGGTGCTGTAGGAgTGAACGTCTGGTTCAGCAGATTTGGCGCAACTGGC
S.f.          CCTGaTTTGGTGCTGTAGGAGCGAACGTCTGGTTCAGCAGATTTGGCGCAACTGGC

NT14          AGATTGTGGTTCGAGTTCGTAGTCGCTCTGAACTTGCGTT-CTGCTTACAGCGTAG
E.c. 1        AGATTGTGGTTCGGGTTCGTAGTCGCTCTGAaCTTGCGTTTCTGCTTACAGCGTAS
E.c. 2        AGATTGTGGTTCGGGTTCGTAGTCGCTCTGAaCTTGCGTTTCTGCTTACAGCGTAS
S.f.          AGATTGTGGTTCGAGTTCGTAGTCGCTCTGAaCTTGCGTTTCTGCTTACAGTGTAG

NT14          -CTTAGCTCCTTACGAAGACGTGCCAGTCGGTCACGACCAACGATGATGCCATTCT
E.c. 1        -CTTAGCTCCTTACGAAGACGTGCCAGTCGGTCACG-CCAACGATGATGCCATTCT
E.c. 2        -CTTAGCTCCTTACGAAGACGTGCCAGTCGGTCACG-CCAACGATGATGCCATTCT
S.f.          CCTTAGCTCCTTACGAAGACGTGCCAGTCGGTCACG-CCAACGATGATGCCATTCT
Probe 437                                          5' CGATGATGCCATTCT

NT14          CTGCCAGCTCCGTCTGG-AGCCGCCGGGTT-CCATATGTTTCGCGAGTGCGGATAT
E.c. 1        CTGCCAGCTCCGTCTGG-AGCCGCCGGGTT-CCATATGTTDcGCRAGTGCGGATAT
E.c. 2        CTGCCAGCTCCGTCTGG-AGCCGCCGGGTT-CCATATGTTDNGCRAGtGCGGATAT
S.f.          CTGCCAGCTCCGTCTGG-AGCCGCCGGGTT-CCATATGTTNcGCRAGTGCGGATAT
437 cont'd    CTGCCAGCTCCGTCTGGGAGCCGCCGGGTTTCC 3'
```

FIGURE 4E

```
NT14        GTGCCACCTTAATCTCCAGTTTTAGCCGCTCATCACTTTGTTTTCTGTCTGAGGGT
E.c. 1      GTGCCACCTTAATCTCCAGTTTDAGCCGCTCATCACTTTGTTDTCTGTCTGAGGGt
E.c. 2      GTGCCACCTTAATCTCCAGTTTDAGCCGCTCATCACTTTGTTDTCTGTCTGAGGGT
S.f.        GTGCCACCTTAATCTCCAGTTTDAGCCGCTCATCACTTTGTTDTCTGTCTGAGGGT

NT14        TCATGCTGTACCCAGTTGTAATAACCGCTCCTGGATACACCAAATAC-TGACACAT
E.c. 1      tCATGCTGTACCCAGTTGTAATAACCGCTCCTGGATACACCAAATACCTGACACAT
E.c. 2      TCATGCTGTACCCAGTTGTAATAACCGCTCCTGGATACACCAAATACCTGACACAT
S.f.        TCATGCTGTACCCAGTTGTAATAACCGCTCCTGGATACACCAAATACCTGACACAT

NT14        CGCTTCAATGGGAAATTGTTGTCGCCATTGTTCGATTAACGCGTATTTTTCAGCGA
E.c. 1      CGCTTSAATDDDAAATTGTTGTCGCCATTGTTCGATTAACGCG-------CAGCGA
E.c. 2      CGCTTNAATGGGAAATTGTTGTCGCCATTGTTCGATTAACGCGnATTTTTCAGCGA
S.f.        CGCTTSAATGGGAAATTGTTrTCGCCATTGTTCGATTAACGCG----------A

NT14        CTCCTGTGCAAAATACGCTGTTGCTTTTTTTAATATATCTCGCTCAAGGCGAGCTT
E.c. 1      CTCCTGTGCAAAATACGCTGTTGCTtTTTTtAATATATCTCGCTCaAGGCGAGCTT
E.c. 2      CTCCTGTGCAAAATACGCTGTTGCTTTTTTTNATATATCTCGCTCAAGGCGAGCTT
S.f.        CTCCTGTGCAAAATACGCTGTTGCTTTTTTNNATATATCTCGCTCaAGGCGAGCTT

NT14        CATTTAACGCCTTACGCAGTCGCAGAATTTCAGATTCCAGTTCAGCCACCGTGCGG
E.c. 1      CATTTAACGCTTtACGCAGTTGCAGAATTTcAGATTCCAGTTcAGCCACCGTGCGG
E.c. 2      CATTTAACGCCTTACGCAGTTGCAGAATTTcAGATTCCAGTTcAGCCACCGTGCGG
S.f.        CATTTAACGCCTTACGCAGTTGCAGAATTTcAGATTCCAGTTcAGCCACCGTGCGG
```

FIGURE 4F

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NT14        GAACCAGGAGTACCGAGCCCTTTTCTGGCGGCGGTAACCCATTGTCCTAAAGTGCC
E.c. 1      GAaCCAGGAGTACCGAGCCCTTTTCTGGCGGCGGTAaCCCATTGTCCTAAAGTGCC
E.c. 2      GAaCCAGGAGTACCGAGCCCTTTTCTGGCGGCGGTAaCCCATTGTCCTAAAGTGCC
S.f.        GAaCCAGGAGTACCGAGCCCTTTTCTGGCGGCGGTAaCCCATTGTCCTAAAGTGCC

NT14        TTCAGGAAGAGATAATCGGGAAGCGCCTTCACTGATC
E.c. 1      TTCAGGAAGAGaTAATCGGGAAGCGCCTTCGCTGATCGAAAGTTGATTTTCAAGAA
E.c. 2      TTCAGGAAGAGnTAATCGGGAAGCGCCTTCGCTGATCGAAAGTTGATTTTCAAGAA
S.f.        TTCAGGAAGAGaTAATCGGGAAGCGCCTTCACTGATCGAAAGTTGATTTTCAGGAA

E.c. 1      CCGTTCTGACAGCTTCGGCTTTGAACTCTgT
E.c. 2      CCGTTCTGACAGCTTCGGCTTTGAACTCTTTAGAGTAACGTTGGTTTTTTCTGCTC
S.f.        CCGTTCTGaCAGCTTCGGCTTTGAACTCTTKAGAGtAACGTTGGGTTTTTCTGCTC

E.c. 2      ATTATTAGCTCCTTCTGATGCCATTCTATTTCAGGAAGGAGTGTCCGTTAAACTCA
S.f.        ATTATTAGCTCCTTCTGATGCCATTCTATTTCAGGAAGGAGTGTCCGTTAAACTCA

E.c. 2      GGCTACCTCAAGATAAAGTTATTAATTTCGAAGATCACATCTTCAATAGGTTTGCG
S.f.        GGCTACCTCAGTGTGATCGGCGATAAGCCCAGaACTCCGCTCCCAGACCTCCCTGC
                      ***
              Right end of repeat.

E.c. 2      GTCCATATTATC
S.f.        CAAAAGCAAAACCG
```

3′

FIGURE 5A

	5′
NT18-1a	ATCTCCTTATGTTATGGAGATTATTAAAAAGAATAACATTAGCGCTCTCGAACT
NT18-1a cont′d	GCATCGTGCAATTGTTGAGTTGAGTAAAAATATGAAGTCGATTGATGATAATGC
NT18-1a cont′d	CAGTAAGAAAAACGACAAGTCATCATTGTATGTATCATGGACTCTGAGTTTTAC
NT18-1a cont′d	TGCTCCAACAAGTAAAGAAGCTCACGATGTGTTGTCTGGGTATATTAATTATGT
NT18-1a cont′d	TTCTTCCCTTGTTGTAAGGGATTTGATGGAAGATATAAGAAATAAACTAGAAGT
NT18-1a cont′d	TAAAACTAATGTTGAAAAAGAAATTCTTGCACTGGATGAGATAAAAATTAGAAA
NT18-1a cont′d	CCAGCTGAATGCAGATATTCGACNCCTCAATTATTCACTGGAGGTTGCTAATGC
NT18-1a cont′d	GGCTGGAATAAAAAAACCTGTATACAGCAATGGTCAGATTATGAAGGATGACCC

FIGURE 5B

NT18-1a cont'd AGATTTTCCTGTGGCTCTCGGTTCTGATGGTATAGCAACTAAATTGAACATCAA
Probe 1712 CCTGTGGCTCTCGGTTCTGATGGTATAGCAACTAAAT

NT18-1a cont'd AAAATCAATCAAGGATGTTTCGGAATTGAGTGGGGAGTTGCGAAATCGTCAATA
Probe 1713 CAAGGATGTTTCGGAATTGAGTGGGGAGTTGCGAAAT

NT18-1a cont'd TGTTGTGAATCAATTGGTTGTGGCGAAAGNGGGGGANGNNGANNNNANGCMANN

NT18-1a cont'd NCAGNANCAANNGTGCCCAACGNNACCGGNCAgaAA
3'

FIGURE 6A

```
                    5'
NT19-2              CCACCACATTGATTGTCTGCCTGAAATAACACGAAAGGCACtGCGTGAACGCTA

NT19-2 cont'd       TGTGGAACAGCTGGTGGCTACAGAGAACAATGTTTCTGAAGTGAAAGCTGTTAC

NT19-2 cont'd       CAGAAAAACACGCAATCCTGACGCTGTCCAGGCAATCGAAGCATATCGCGGTTC
Probe 1684                                      CAGGCAATCGAAGCATATCGCGGTTC

NT19-2 cont'd       TCCACAACTGATGGAAGAACGCCTGAATGCGCTGACCGAAAACCAGCGCTGGGT
1684   cont'd       TCCACAACT
Probe 1685                                          TGAATGCGCTGACCGAAAACCAGCGCTGGGT

NT19-2 cont'd       ATCTGAAGCAAGAGCTGCGCTGGTGGTGGAAGTGCTGAAGCTGGAAAGCGCCGG

1685 cont'd         ATCT
```

FIGURE 6B

NT19-2 cont'd TAACCCCGGGCGACTGAAAGCCATTAACTTTCTTGTTGAAAAAGCCCCTAAAGG

NT19-2 cont'd TGAGCTGCCGGAGCGCCTGCAACAGGCCGCAGTTAACGCCAATGCAAAACGTGG

NT19-2 cont'd CGCTAATCGT 3'

FIGURE 7

```
                        5′
S.d. ompA               CTGACGACCTGGACGTGTACACTCGTCTGGGTGGTATGGTTTGGCGTGCAGA
E.c. ompA               CTGACGACCTGGACATCTACACTCGTCTGGGTGGCATGGTATGGCGTGCAGA


S.d. ompA cont'd        CACCAAAGCTCACAACAATGTGACAGGTGAATCTGAGAAAAACCACGATACC
E.c. ompA cont'd        CACTAAATCC-------AACGTTTATGGT----------AAAAACCACGACACC
Probe 1707            3′ GGTTTCGAGTGTTGTTACACTGTCCACTTAGACTC  5′


S.d. ompA cont'd        GGCGTTTCTCCGGTATTCGCAGGTGGTGTTGAATGGGCCATCACTCCTGAAA
E.c. ompA cont'd        GGCGTTTCTCCGGTCTTCGCTGGCGGTGTTGAGTACGCGATCACTCCTGAAA
Probe 1706                    3′ GGCCATAAGCGTCCACCACAACTTACCCGGTAGTG  5′


                                                              3′
S.d. ompA cont'd        TCGCTACCCGTCTGGAATACCAGTGGAC
E.c. ompA cont'd        TCGCTACCCGTCTGGAATACCAGTGGAC
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: NUCLEIC ACID PROBES FOR THE DETECTION OF SHIGELLA

(57) Abstract

The invention relates to methods of detection of bacteria of the genus Shigella and/or Enteroinvasive *E. coli* (EIEC) by use of a set of nucleic acid probes. The invention further relates to a set of Shigella specific chromosomal sequences and fragments and to probes derived from the Shigella specific fragments. Additionally, probes were derived from a sequence from the Shigella ompA gene. In particular, a series of probes, each approximately 40 nucleotides in length, were designed having specificity for Shigella or for Shigella and Enteroinvasive *E. coli*, and having utility in nonisotopic test formats which require amplification to achieve high sensitivity. Specific hybridization probe sets which are capable of detecting substantially all clinically significant serotypes of Shigella, as well as enteroinvasive strains of *E. coli*, are disclosed.

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INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/US 92/06617

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12Q 1/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DATA BASE: MEDLINE, BIOTECHNOLOGY ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, File 154, Medline, Dialog accession no. 07171566, Medline accession no. 90078566, Venkatesan MM et al: "Use of Shigella flexneri ipaC and ipaH gene sequences for the general identification of Shigella spp. and enteroinvasive Escherichia coli", J Clin Microbiol Dec 1989, 27 (12) p 2687-91	1
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X	Dialog Information Services, File 154, Medline, Dialog accession no. 07673589, Medline accession no. 91192589, Cleuziat P et al: "Specific detection of Escherichia coli and Shigella species using fragments of genes coding for beta-glucuronidase", FEMS Microbiol Lett Nov 1990, 60 (3) p 315-22	1
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
2 February 1993	25. 02. 93
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer MIKAEL G:SON BERGSTRAND

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/06617

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A2, 0357306 (INTEGRATED GENETICS, INC.), 7 March 1990 (07.03.90)	1-44
	--	
A	US, A, 4816389 (SANSONETTI ET AL), 28 March 1989 (28.03.89)	1-44
	--	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06617

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The claimed invention relates to DNA fragments derived from the genus Shigella that are useful when detecting Shigella and/or Enteroinvasive E. coli. Both the problem (Detecting Shigella bacteria) and the solution (Detecting Shigella bacteria by using DNA probes derived from the Shigella chromosome) are known in the art. This leads to the following regrouping:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

1. Claims 1,4,7-10,17-21, and 39-44: Probes derived from the chromosome of Shigella flexneri and probe compositions comprising these probes.
2. Claims 2-3,5-6,12-38: Probes derived from the chromosome of Shigella sonnei and probe compositions comprising these probes.
3. Claims 11 and 17-26: Probes derived from the chromosome of Shigella dysenteriae and probe compositions comprising these probes.

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INTERNAT AL SEARCH REPORT
Information ... patent family members

International application No.
08/01/93 PCT/US 92/06617

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US-A- 5084565	28/01/92
US-A- 4816389	28/03/89	EP-A,B- 0170584	05/02/86
		FR-A,B- 2567541	17/01/86
		JP-A- 61044000	03/03/86
		US-A- 4992364	12/02/91